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16. ABSTRACT

This report considers the large number of chemicals which contain at least one nitro substituent on an aromatic ring. Approximately 250-300 chemicals are listed as commercial nitroaromatic compounds. However, only about 40 compounds are produced or consumed annually in quantities over 500,000 pounds and perhaps another 50-100 compounds exceed 100,000 pounds. Nitroaromatic compounds are used as pesticides, perfumes, explosives, and chemical intermediates. This report focuses upon the non-pesticidal nitroaromatics. Because of the large number of compounds considered in this report, comprehensive information on individual compounds could not be developed. However, adequate information is available to provide priorities for further study and research. Production volume, uses, environmental fate, monitoring, and biological effects were considered. In general, nitroaromatic compounds appear to be fairly persistent and exhibit either hematologic or metabolic effects at high levels of exposure. Most of the large-volume nitroaromatics have not been screened for carcinogenic, mutagenic, or teratogenic effects.

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EXECUTIVE SUMMARY

This report considers the large number of chemicals which contain at least one nitro substituent on an aromatic ring. Approximately 250-300 chemicals are listed as commercial nitroaromatic compounds. However, only about 40 compounds are produced or consumed annually in quantities over 500,000 pounds and perhaps another 50-100 compounds exceed 100,000 pounds. Nitroaromatic compounds are used as pesticides, perfumes, explosives, and chemical intermediates. This report focuses upon the non-pesticidal nitroaromatics.

Because of the large number of compounds considered in this report, comprehensive information on individual compounds could not be developed. However, adequate information is available to provide priorities for further study and research. Production volume, uses, environmental fate, monitoring, and biological effects were considered. In general, nitroaromatic compounds appear to be fairly persistent and exhibit either hematologic or metabolic effects at high levels of exposure. Most of the large-volume nitroaromatics have not been screened for carcinogenic, mutagenic, or teratogenic effects. Nevertheless, the following compounds appear to have high contamination potential: nitrobenzene (655 million lbs/year; detected in drinking water); 2,4- (and 2,6-) dinitrotoluene (471 million lbs/year; detected in drinking water); 2,4,6-trinitrotoluene (TNT) (432 million lbs/year; demonstrated to have considerable pollution problems; not detected in drinking water; may be biodegradable); o-, m-, p-chloronitrobenzene (60, 110, and 8 million lbs/year, respectively; persistent; meta-isomer detected in drinking water); and 1,3-dinitrobenzene (12 million lbs/year; persistent; detected in drinking water). For a more detailed list of chemicals, see the Summary and Conclusions in Section V, p. 499.

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Investigation of Selected Potential Environmental Contaminants:
Nitroaromatic Compounds

Introduction

This report reviews the environmental hazard involved in the commercial use of an important and large group of chemicals, nitroaromatics. Information on chemical and physical properties, production, uses, environmental contamination potential, and biological effects of the compounds is reviewed. The group includes any compound that has an aromatic system with a nitro group directly attached to the aromatic ring. Most of the compounds are substituted benzenes, but naphthalene and other aromatic systems are frequently encountered. For the most part, nitroaromatics have direct uses or are used as chemical intermediates in the explosive, dye, pigment, pharmaceutical, rubber, pesticide, and perfume industries.

Because of the large number of compounds considered in this report, only limited information on any individual chemical can be presented. For this reason, pesticide nitroaromatic compounds have not been as thoroughly treated as they could be from the available information. The degree of detail for the other chemicals was frequently dependent upon the commercial importance of the compounds, although the availability of information was often a major factor.

The nitroaromatic compounds that are produced in commercial quantities ($1,000 or a 1,000 lbs per year) are listed with the name of the producer(s) in the Chemical Index. Other nitroaromatics that do not appear to be commercial products but for which information is available are also listed in the Appendix, and that appendix can be used as an index to information on individual compounds.

I. Physical and Chemical Data

A. Structure and Properties

1. Chemical Structure

Nitroaromatic compounds have at least one nitro substituent attached to an aromatic ring. The nitrogen and two oxygens of the nitro group are kept a large percentage of the time in the same plane as the aromatic ring by conjugation between the sp^2 orbitals of the ring and the nitro group (see Figure 1).

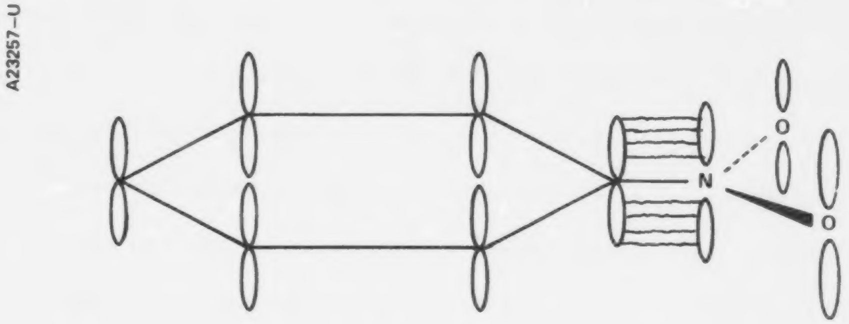
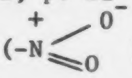


Figure 1. Conjugative Interaction Between Aromatic Systems and Nitro Functional Groups

This conjugative interaction between the nitro substituent and the aromatic ring has considerable impact on the chemical reactions that the compound may undergo, and affects the electron (UV) spectra of the chemicals (See Section I-B, p. 21 for examples). The nitro functional group is polar and electrophilic ( $-\overset{+}{N}(=O)O^-$), and this results in inductive withdrawal of electrons from the ring through the σ bond (deactivates the ring to electrophilic substitution) and a high dipole moment.

2. Physical Properties

The physical properties of 96 important nitroaromatic compounds are presented in Table 1. The compounds in the table were selected because (1) production data were available or (2) environmental fate or biological effects data were available and the compound was listed as a commercial product. The data presented in Table 1 illustrate that the physical properties are very dependent upon the type, number, and position of the substituents.

In general, nitroaromatic compounds are not very soluble in water. Water solubility usually decreases with increasing nitro substitution. Other substituents, like chlorine groups, can have considerable effects upon the water solubility. For example, Eckert (1962) has shown that the water solubility of chloronitrobenzenes decreases with an increase in the degree of chlorination (See Table 2). In contrast, groups such as amino, hydroxyl, carboxyl, etc. increase the water solubility of nitroaromatic compounds.

Table 2. Water Solubility of Chlorine Substituted Nitrobenzenes (Eckert, 1962)

Compound	# Cℓ	Water Solubility μM/liter, 20°C
Nitrobenzene	0	15,100
4-Chloronitrobenzene	1	2,877
2-Chloronitrobenzene	1	2,800
3-Chloronitrobenzene	1	1,732
1-Chloro-2,4-dinitrobenzene	1	--
1,2-Dichloro-4-nitrobenzene	2	679
1,4-Dichloro-2-nitrobenzene	2	480
1,2,4,5-Tetrachloro-3-nitrobenzene	4	8
Pentachloronitrobenzene	5	1.5

Table 1. Physical Properties of Significant Nitroaromatic Chemicals

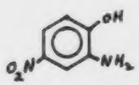
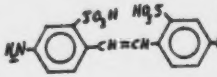
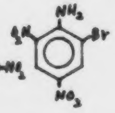
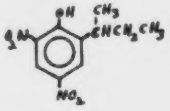
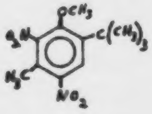
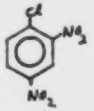
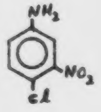
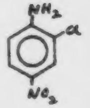
Compound	2-Amino-4-nitrophenol	4-Amino-4'-nitro-2,2'-stilbene-disulfonic acid	2-Bromo-4,6-dinitroaniline	2-sec-Butyl-4,6-dinitrophenol	6-tert-Butyl-3-methyl-2,4-dinitroanisole	1-Chloro-2,4-dinitrobenzene	4-Chloro-3-nitroaniline	2-Chloro-4-nitroaniline
Synonym	2-hydroxy-5-nitroaniline 4-nitro-2-amino-1-hydroxybenzene		6-bromo-2,4-dinitroaniline	4,6-dinitro-o-sec-butylphenol 2,4-dinitro-6-sec-butylphenol DNOSBP DNSBP DNBP	4-tert-butyl-3-methoxy-2,6-dinitrotoluene Musk ambrette	2,4-dinitro-1-chlorobenzene DNCB	3-nitro-chloraniline	1-amino-2-chloro-4-nitrobenzene o-chloro-p-nitroaniline OCPNA
Chemical Abstract Registry No.	99-57-0		1817-73-8	88-85-7	83-66-9	97-00-7	635-22-3	121-87-9
Formula	$C_6H_6N_2O_3$	$C_{14}H_{12}N_2O_8S_2$	$C_6H_4BrN_3O_4$	$C_{10}H_{12}N_2O_5$	$C_{12}H_{16}N_2O_5$	$C_6H_3ClN_2O_4$	$C_6H_5ClN_2O_2$	$C_6H_5ClN_2O_2$
Structure								
Molecular Weight	154.12	400.24	262.03	241.11	268	202.56	172.58	172.58
Melting Point (°C)	80-90 (1 mol. H_2O) 143 anhydrous		153-4	42		53.4 (alpha, stable) 43 (beta) 27 (gamma)	102-3	104-5
Boiling Point (°C)	--		sublimes			315		
Specific Gravity or Density								
Water Solubility (g/100g)				0.073 (250)-25°		insoluble		
Vapor Pressure (mmHg)								
Octanol-Water Partition Coefficient (log of)								
Ultraviolet Spectral Data	λmax 373, 308 εmax 4250, 4780		336, 268, 229 12000, 8700, 10100		265	206, 238		

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

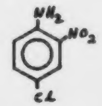
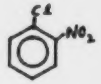
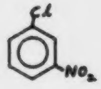
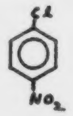
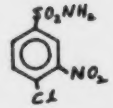
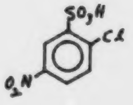
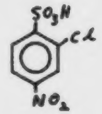
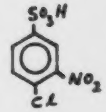
Compound	4-Chloro-2-nitroaniline	1-Chloro-2-nitrobenzene	1-Chloro-3-nitrobenzene	1-Chloro-4-nitrobenzene	4-Chloro-3-nitrobenzenesulfonamide	2-Chloro-5-nitrobenzenesulfonic acid	2-Chloro-4-nitrobenzenesulfonic acid	4-Chloro-3-nitrobenzenesulfonic acid
Synonym	1-amino-4-chloro-2-nitrobenzene p-chloro-o-nitroaniline Azoic diazo component 9 CI #37040	chloro-o-nitrobenzene o-nitrochlorobenzene ONCB o-nitrophenyl chloride	m-nitrochlorobenzene	p-chloronitrobenzene 1-nitro-4-chlorobenzene PNCB p-nitrophenyl chloride	4-chloro-3-nitrosulfamylbenzene 1-chloro-2-nitro-4-sulfonamidobenzene o-nitrochlorobenzene sulfonamide Yellow sulfone	p-nitrochlorobenzene-o-sulfonic acid 2-chloro-5-nitrosulfobenzene		
Chemical Abstract Registry No.	89-63-4	88-73-3	121-73-3	100-00-5	97-09-6			
Formula	$C_6H_5ClN_2O_2$	$C_6H_4ClNO_2$	$C_6H_4ClNO_2$	$C_6H_4ClNO_2$	$C_6H_5ClN_2O_4S$	$C_6H_4ClNO_5S$	$C_6H_4ClNO_5S$	$C_6H_4ClNO_5S$
Structure								
Molecular Weight	172.58	157.6	157.6	157.6	236.4	237.43	237.43	237.43
Melting Point (°C)	116-7	35.5 32.2 (HCP)	24 (unstable) 44	83	172-3			72
Boiling Point (°C)		245	235-6	242				
Specific Gravity or Density		1.368	1.534	1.520				
Water Solubility (g/100g)								
Vapor Pressure (mmHg)		10 mm Hg (230°F)						
Octanol-Water Partition Coefficient (log of)		2.24	2.41	2.41				
Ultraviolet Spectral Data λmax	234.5		257, 206	270	292, 222.5			
εmax					814, 20300			

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

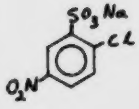
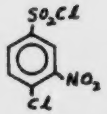
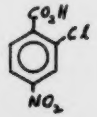
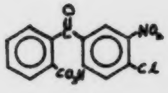
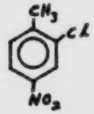
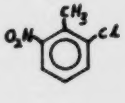
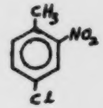
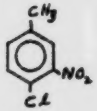
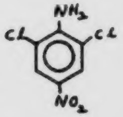
Compound	2-Chloro-5-nitrobenzenesulfonic acid, sodium salt	4-Chloro-3-nitrobenzenesulfonyl chloride	2-Chloro-4-nitrobenzoic acid	o-(4-Chloro-3-nitrobenzoyl) benzoic acid	2-Chloro-4-nitrotoluene	2-Chloro-6-nitrotoluene	4-Chloro-2-nitrotoluene	4-Chloro-3-nitrotoluene	2,6-Dichloro-4-nitroaniline
Synonym				-carboxy-4'-chloro-3'nitrobenzophenone	o-chloro-p-nitrotoluene				1-amino-2,6-dichloro-4-nitrobenzene
Chemical Abstract Registry No.	946-30-5	97-08-5	99-60-5	85-54-1	121-86-8	83-42-1	89-59-8	89-60-1	99-30-9
Formula	$C_6H_3ClNO_5NA$	$C_6H_3Cl_2NO_4S$	$C_7H_4ClNO_4$	$C_{14}H_8ClNO_5$	$C_7H_6ClNO_2$	$C_7H_6ClNO_2$	$C_7H_6ClNO_2$	$C_7H_6ClNO_2$	$C_6H_4Cl_2N_2O_2$
Structure									
Molecular Weight	259.6	256.1	201.57	271.23	171.58	171.58	171.58	171.58	207.03
Melting Point (°C)			140 139-141		65	37	37	7	191
Boiling Point (°C)						236-8	239.5-240 (215.5)		
Specific Gravity or Density							1.2559		
Water Solubility (g/100g)									
Vapor Pressure (mmHg)									
Octanol-Water Partition Coefficient (log of)									
Ultra-violet Spectral Data λmax εmax		345, 222	262, 209		304, 254	248	300, 251	298, 250	245, 350

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

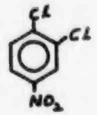
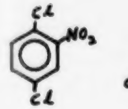
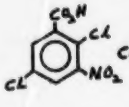
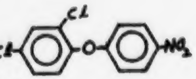
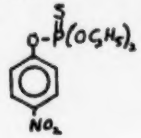
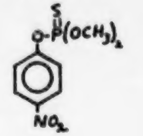
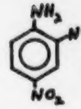
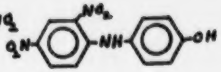
Compound	1,2-Dichloro-4-nitrobenzene	1,4-Dichloro-2-nitrobenzene	2,5-Dichloro-3-nitrobenzoic acid	2,4-Dichlorophenyl-4-nitrophenyl ether	0,0-Diethyl-p-nitrophenyl-phosphorothioate	0,0-Dimethyl-p-nitrophenyl-phosphorothioate	2,4-Dinitroaniline	p-(2,4-Dinitroanilino)phenol
Synonyms		2,5-dichloronitrobenzene	Dichloronitrobenzoic acid, isomeric mixture	Nitrofen NIP	0,0,diethyl-0-(p-nitrophenyl)thionophosphate Ethyl parathion	0,0-dimethyl-o-(p-nitrophenyl) thionophosphate Methyl parathion Paridol MPT	1-amino-2,4-dinitrobenzene	
Chemical Abstract Registry No.	99-54-7	89-61-2	88-86-8	1836-75-5	56-38-2	298-00-0	97-02-9	119-15-3
Formula	$C_6H_3Cl_2NO_2$	$C_6H_3Cl_2NO_2$	$C_7H_3Cl_2NO_4$	$C_{12}H_7Cl_2NO_3$	$C_{10}H_{14}NO_5PS$	$C_8H_{10}NO_5PS$	$C_6H_5N_3O_4$	$C_{12}H_9N_3O_5$
Structure								
Molecular Weight	192.0	192.0	236.02	284.1	291.3	263.2	183.12	275.2
Melting Point (°C)	43	56		71-2			187-8	196
Boiling Point (°C)	255-6	266						
Specific Gravity or Density	1.4558	1.669						
Water Solubility (g/100g)	insoluble							
Vapor Pressure (mmHg)								
Octanol-Water Partition Coefficient (log of)								
Ultraviolet Spectral Data λmax	266, 212.5	299, 219.7		293			336, 257, 225	224
εmax				293				16600

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

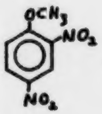
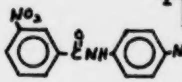
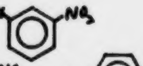
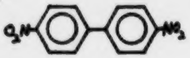
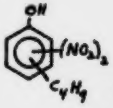
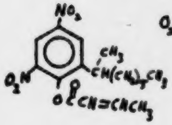
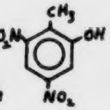
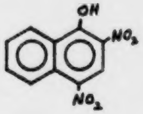
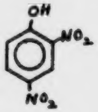
Compound	2,4-Dinitro-anisole	3',4-Dinitro-benzanilide	1,3-Dinitro-benzene	4,4'-Dinitro-biphenyl	Dinitrobutyl-phenol, ammonium salt	Dinitrocaprylphenyl crotonate	4,6-Dinitro-o-cresol	2,4-Dinitro-α-naphthol	2,4-Dinitro-phenol
Synonym	1-methoxy-2,4-dinitrobenzene	3'-nitro-N-(4-nitrophenyl) benzamide	m-dinitro-benzene			2-(1-methylheptyl)-4,6-dinitrophenyl crotonate Karathane, Dinocap	DNC, DNOC 2-methyl-3,5-dinitrophenol 4,6-dinitro-2-hydroxytoluene	2,4-dinitro-1-naphthol Martius yellow	α-dinitro-phenol 1-hydroxy-2,4-dinitrobenzene
Chemical Abstract Registry No.	119-27-7		99-65-0	1528-74-1		39300-45-3	534-52-1	605-69-6	51-28-5
Formula	$C_7H_6N_2O_5$	$C_{13}H_9N_3O_5$	$C_6H_4N_2O_4$	$C_{12}H_8N_2O_4$	$C_{10}H_{16}N_3O_4$	$C_{18}H_{24}N_2O_6$	$C_7H_6N_2O_5$	$C_{10}H_6N_2O_5$	$C_6H_4N_2O_5$
Structure									
Molecular Weight	198.13	287.2	168.1	244.2	242.3	364.4	198.1	234.2	184.11
Melting Point (°C)	95.5-6.0	223-4	89.75	234-5		138-40 6.05 mm.	85, 85.8		114
Boiling Point (°C)			302						
Specific Gravity or Density			1.571						1.683
Water Solubility (g/100g)			0.05 (15°C) 0.32 (100°C)				0.013 (15°)		0.6 (25°) 0.02 (12.5°) 1.32 (100°)
Vapor Pressure (mmHg)							105×10^{-6} (25°C)		
Octanol-Water Partition Coefficient (log of)			1.49						1.51
Ultra-violet Spectral Data λmax	290, 251.5, 213, 266, 235			305			263		
εmax	10900, 7430, 14000			25900					

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

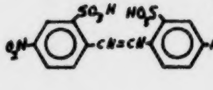
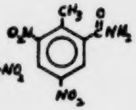
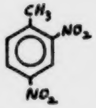
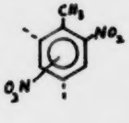
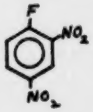
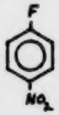
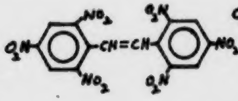
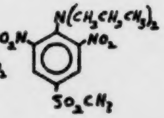
Compound	4,4'-Dinitrostilbene-2,2'-disulfonic acid	3,5-Dinitrotoluamide	2,4-Dinitrotoluene	2,4-(and 2,6-) Dinitrotoluene	1-Fluoro-2,4-dinitrobenzene	p-Fluoronitrobenzene	2,2',4,4',6,6'-Hexanitrostilbene	4-(Methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline
Synonym	2,2'-(1,2-ethanediyl) bis [5-nitro] benzenesulfonic acid	2-methyl-3,5-dinitrobenzamide Zoalene	2,4-dinitrotoluol 1-methyl-2,4-dinitrobenzene		2,4-dinitro-1-fluorobenzene	1-fluoro-4-nitrobenzene		Nitralin
Chemical Abstract Registry No.	128-42-7	148-01-6	121-14-2	121-14-2 606-20-2	70-34-8	350-46-9	20062-22-0	4726-14-1
Formula	$C_{14}H_{10}N_2O_{10}S_2$	$C_8H_7N_3O_5$	$C_7H_6N_2O_4$	$C_7H_6N_2O_4$	$C_6H_3FN_2O_4$	$C_6H_4FNO_2$	$C_{14}H_6N_6O_{12}$	$C_{13}H_{19}N_3O_6S$
Structure								
Molecular Weight	430.4	225.13	182.1	182.1	186.1	141.0	450.2	345.4
Melting Point (°C)			69.5-70.5 71-2					
Boiling Point (°C)			decomposes at 300					
Specific Gravity or Density			1.521					
Water Solubility (g/100g)			0.027g/100ml (22°)					0.6
Vapor Pressure (mmHg)								1.5×10^{-6} (25°)
Octanol-Water Partition Coefficient (log of)								
Ultraviolet Spectral Data λmax εmax			234			256.5		

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

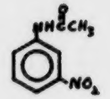
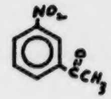
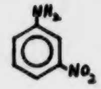
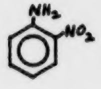
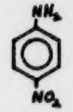
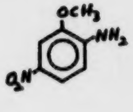
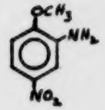
Compound	3'-Nitroacetanilide	3'-Nitro-acetophenone	m-Nitroaniline	o-Nitroaniline	p-Nitroaniline	4-Nitro-o-anisidine	5-Nitro-o-anisidine
Synonym	m-acetylamino-nitrobenzene N-acetyl-m-nitroaniline N-(m-nitrophenyl) acetamide m-Nitroacetanilide		3-nitroaniline	1-amino-2-nitrobenzene 2-nitroaniline Azoic diazo component 6 2-nitrobenzenamine	1-amino-4-nitrobenzene 4-nitroaniline	2-amino-5-nitroanisole 2-methoxy-4-nitroaniline Azoic diazo component 5 (salt) CI #37125 (salt)	2-amino-4-nitroanisole 2-methoxy-5-nitroaniline o-anisidine nitrate Azoic diazo component 13 (salt) CI #37130 (salt)
Chemical Abstract Registry No.	122-28-1	121-89-1	99-09-2	88-74-4	100-01-6	97-52-9	99-59-2
Formula	$C_8H_8N_2O_3$	$C_8H_7NO_3$	$C_6H_6N_2O_2$	$C_6H_6N_2O_2$	$C_6H_6N_2O_2$	$C_7H_8N_2O_3$	$C_7H_8N_2O_3$
Structure							
Molecular Weight	180.0	165	138.12	138.12	138.12	168.2	168.2
Melting Point (°C)		76-8	114	71-2	146-8	139-140	
Boiling Point (°C)			286	270 with decomposition	200°C (10 mmHg)		
Specific Gravity or Density					1.437		
Water Solubility (g/100g)			0.11	0.1	0.08		
Vapor Pressure (mmHg)							
Octanol-Water Partition Coefficient (log of)			1.46	1.79	1.19		
Ultraviolet Spectral Data λmax εmax		225.5	371, 233 1091, 12000	277, 232		388, 258 14700, 5580	374, 303, 257, 227 3900, 5300, 14200, 11300

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

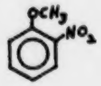
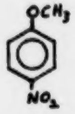
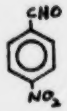
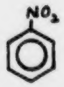
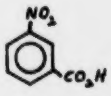
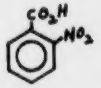
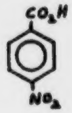
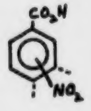
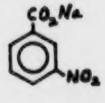
Compound	o-Nitroanisole	p-Nitroanisole	p-Nitrobenzaldehyde	Nitrobenzene	m-Nitrobenzoic acid	o-Nitrobenzoic acid	p-Nitrobenzoic acid	m- and p-Nitrobenzoic acids	m-Nitrobenzoic acid, sodium salt
Synonym	2-nitroanisole 2-methoxynitrobenzene	4-nitroanisole 4-methoxynitrobenzene			3-nitrobenzoic acid	2-nitrobenzoic acid	4-nitrobenzoic acid 4-nitrodracylic acid		
Chemical Abstract Registry No.	91-23-6	100-17-4	555-16-8	98-95-3	121-92-6	552-16-9	62-23-7		827-95-2
Formula	$C_7H_7NO_3$	$C_7H_7NO_3$	$C_7H_5NO_3$	$C_6H_5NO_2$	$C_7H_5NO_4$	$C_7H_5NO_4$	$C_7H_5NO_4$	$C_7H_5NO_4$	$C_7H_4NaNO_4$
Structure									
Molecular Weight	153.1	153.1	151.12	123.1	167.1	167.1	167.1	167.1	189
Melting Point (°C)	9.4	54	42-44	5.1	141.4	147.5 (144-5)	242.4		>300
Boiling Point (°C)	272.3	274	153 (23 mmHg)	210.9					
Specific Gravity or Density				1.20[illegible]					
Water Solubility (g/100g)	0.17 (30°)	0.06 (30°)	0.23	0.1					
Vapor Pressure (mmHg)				1 (44°C) 10 (85.4°C)					
Octanol-Water Partition Coefficient (log of)				1.85		1.31	1.85		
Ultraviolet Spectral Data λmax	321, [illegible]	304	26[illegible]	259	[illegible]5.4		259		260, 211
εmax			10600	7180					7870, 21000

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

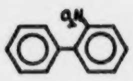
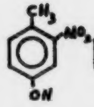
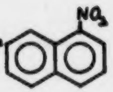
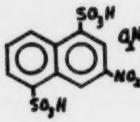
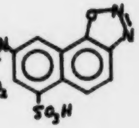
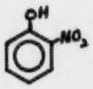
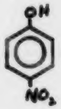
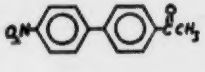
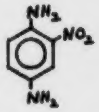
Compound	o-Nitrobiphenyl	2-Nitro-p-cresol	1-Nitronaphthalene	3-Nitro-1,5-naphthalenedisulfonic acid	7- (and 8-) Nitronaph (1,2-d)(1,2,3) oxadiazole-5-sulfonic acid	o-Nitrophenol	p-Nitrophenol	4'-(p-Nitrophenyl)acetophenone	2-Nitro-p-phenylenediamine
Synonym				nitro casella acid	7-nitrodiazo acid	2-hydroxynitrobenzene			4-amino-2-nitroaniline 1,4-diamino-2-nitrobenzene
Chemical Abstract Registry No.	86-00-0	119-33-5	86-57-7	117-86-2		88-75-5	100-02-7	135-69-3	5307-14-2
Formula	$C_{12}H_9NO_2$	$C_7H_7NO_3$	$C_{10}H_7NO_2$	$C_{10}H_7NO_8S_2$	$C_{10}H_5N_3O_6S$	$C_6H_5NO_3$	$C_6H_5NO_3$	$C_{14}H_{11}NO_3$	$C_6H_7N_3O_2$
Structure									
Molecular Weight	199.21	153.1	173.2	333.3	295.2	139.1	139.1	241	153.1
Melting Point (°C)			57.8			44-45	114	220	
Boiling Point (°C)	325		304			214-216	279, decomposes		
Specific Gravity or Density						1.2942	1.479		
Water Solubility (g/100g)						0.2	0.804 (15°) 1.6 (25°) 29.1 (90°)		
Vapor Pressure (mmHg)						1 mm. Hg (49.3°)			
Octanol-Water Partition Coefficient (log of)						1.79	1.91		
Ultraviolet Spectral Data λmax	231	276, 214	331, 212.5			345, 272	31	530, 340, 287, 275, 216	441, 231
εmax						3330, 6300			

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

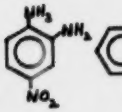
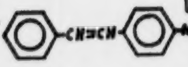
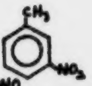
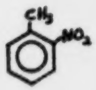
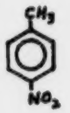
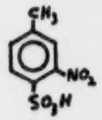
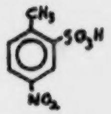
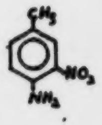
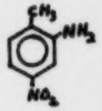
Compound	4-Nitro-o-phenylenediamine	4-Nitrostilbene	3-Nitrotoluene	2-Nitrotoluene	4-Nitrotoluene	3-Nitro-p-toluenesulfonic acid	4-Nitro-o-toluene-sulfonic acid	3-Nitro-p-toluidine	4-Nitro-o-toluidine
Synonym	2-amino-4-nitroaniline 1,2-diamino-4-nitrobenzene		m-nitrotoluene 3-methylnitrobenzene 3-nitrotoluol 1-methyl-3-nitrobenzene	2-methylnitrobenzene 1-methyl-2-nitrobenzene o-nitrotoluene	4-methylnitrobenzene 1-methyl-4-nitrobenzene 4-nitrotoluol p-nitrotoluene		1-methyl-4-nitro-2-sulfobenzene 4-nitro-2-sulfotoluene p-nitro-toluene-o-sulfonic acid	4-amino-3-nitrotoluene 4-methyl-2-nitroaniline 3-nitro-4-toluidine Azoic diazo component 8 (salt) CI #37110 (salt)	2-amino-4-nitrotoluene 1-amino-2-methyl-5-nitrobenzene 2-methyl-5-nitroaniline 4-nitro-2-toluidine
Chemical Abstract Registry No.	99-56-9	4003-94-5	99-08-1	88-72-2	99-99-0	97-06-3	121-03-9	119-32-4	99-52-5
Formula	$C_6H_7N_3O_2$	$C_{14}H_{11}NO_2$	$C_7H_7NO_2$	$C_7H_7NO_2$	$C_7H_7NO_2$	$C_7H_7NO_5S$	$C_7H_7NO_5S$	$C_7H_8N_2O_2$	$C_7H_8N_2O_2$
Structure									
Molecular Weight	153.1	226.3	137.1	137.1		217.2	217.2	152.15	152.15
Melting Point (°C)	201-2		16.1	-10.5*, -4.18 (stable)	51.9		133.5 anydrous, 130 (+ 2 mol. H_2O)	77-8	129-132
Boiling Point (°C)			232.6	221.7	238.3				
Specific Gravity or Density			1.1618	1.1643	1.1226				
Water Solubility (g/100g)			0.0498 (30°)	0.0652 (30°C)	0.004g/100ml (15°)				
Vapor Pressure (mmHg)			1 (50°C)	1 mm Hg 50°C 13 100.2 30 119.2	1 (53.7°C)				
Octanol-Water Partition Coefficient (log of)			2.45	2.30	2.37				
Ultraviolet Spectral Data λmax εmax	265		265 7700	250	264			279.5, 228	225

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

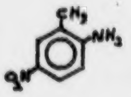
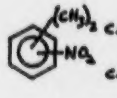
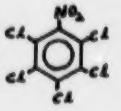
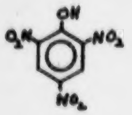
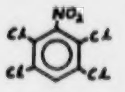
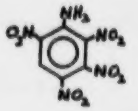
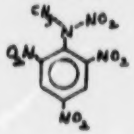
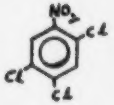
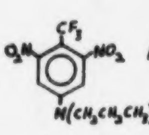
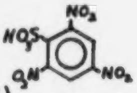
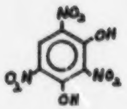
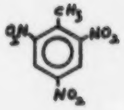
Compound	5-Nitro-o-toluidine	Nitroxylenes	Pentachloronitrobenzene	Picric acid	1,2,4,5-Tetrachloro-3-nitrobenzene	2,3,4,6-Tetranitroaniline	Tetryl
Synonym	2-amino-5-nitrotoluene 4-amino-3-methyl nitrobenzene 2-methyl-4-nitroaniline 5-nitro-2-toluidine		Terrachlor	carbazotic acid 2-hydroxy-1,3,5-trinitrobenzene nitroxanthic acid phenol trinitrate picronitric acid 2,4,6-trinitrophenol	Tecnazene 2,3,5,6-Tetrachloro-1-nitrobenzene	1-amino-2,3,4,6-tetranitrobenzene	N-methyl-N,2,4-tetranitroaniline N-methyl-N-nitro-2,4,6-trinitroaniline methyl 2,4,6-trinitrophenylnitramine
Chemical Abstract Registry No.	99-55-8		82-68-8	88-89-1	117-18-0	3698-54-2	479-45-8
Formula	$C_7H_8N_2O_2$	$C_8H_9NO_2$	$C_6Cl_5NO_2$	$C_6H_3N_3O_7$	$C_6HCl_4NO_2$	$C_6H_3N_5O_8$	$C_7H_5N_5O_8$
Structure							
Molecular Weight	152.15	151.2	295.3	229.1	260.9	273.1	287.15
Melting Point (°C)			146.5	122.5	99 (2,3,5,6) 65-7 (2,3,4,5-)		129.45 with slight decomposition 128.75 (military)
Boiling Point (°C)				decomposes above 300			
Specific Gravity or Density				1.763			
Water Solubility (g/100g)				1.4 (20°C) 6.8 (100°C)			0.005 (0°C) 0.0195 (50°C) 0.184 (100°C)
Vapor Pressure (mmHg)				2mm. Hg 195°C 50mm. Hg 255°C			
Octanol-Water Partition Coefficient (log of)							
Ultraviolet Spectral Data λmax	227		301, 212.5	352	(2,3,5,6-) 296, 208.5 (2,3,4,5-) 205		224
εmax			635, 72900		(2,3,5,6-) 1100, 53400 (2,3,4,5-) 44000		2280

Table 1. Physical Properties of Significant Nitroaromatic Chemicals (Cont'd.)

Compound	1,2,4-Trichloro-5-nitrobenzene	α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine	2,4,6-Trinitrobenzenesulfonic acid	2,4,6-Trinitroresorcinol	2,4,6-Trinitrotoluene
Synonym		Trifluralin Treflan	picryl sulfonic acid	styphnic acid 2,4-dihydroxy-1,3,5-trinitrobenzene	α-trinitrotoluene TNT
Chemical Abstract Registry No.	89-69-0	1582-09-8	2508-19-2	82-71-3	118-96-7
Formula	$C_6H_2Cl_3NO_2$	$C_{13}H_{16}F_3N_3O_4$	$C_6H_3N_3O_9S$	$C_6H_3N_3O_8$	$C_7H_5N_3O_6$
Structure					
Molecular Weight	226.4	335.3	293.2	245.1	227.1
Melting Point (°C)	57	48.5-49		175.5 (180°)	82 (80.2)
Boiling Point (°C)		96-7 (0.18 mm Hg)		sublimes	explodes 240 (varies)
Specific Gravity or Density					
Water Solubility (g/100g)		1 ppm		0.45 (15°C) 0.57 (20°C) 0.68 (25°C) 1.136 (62°C)	0.013
Vapor Pressure (mmHg)		1.99×10^{-4} (24.5°)			0.053 (85°C)
Octanol-Water Partition Coefficient (log of)					
Ultraviolet Spectral Data λmax εmax	305, 260, 22			392, 336, 265, 208 9780, 8820, 12500	

The melting and boiling points generally increase with the number of nitro groups, as does the thermal instability of the compounds. The polynitroaromatic compounds frequently decompose before they melt.

The reported vapor pressures of nitroaromatic compounds are listed in Table 1. Lenchitz and Velicky (1970) used the measured vapor pressure of three nitrotoluenes to calculate the heats of sublimation. They found that the change in the heat of sublimation was not equal for each successive nitro group substitution. They attributed this result to the high polarity of the nitro group, which influences inter- and intramolecular forces.

3. Principal Contaminants and Specifications of Commercial Products

Nitroaromatic compounds are produced by such processes as nitration, sulfonation, chlorination, reduction, and oxidation. Whenever a new substituent is added to the ring, a number of isomers are usually formed. For example, mononitration of toluene will yield approximately 62-63% o-nitrotoluene, 3-4% m-nitrotoluene, and 33-34% p-nitrotoluene, depending upon the nitration conditions (Matsuguma, 1967a). The intended use of the compound determines the degree of purification. For example, dinitrotoluene is available from Du Pont in five different grades: (1) Dinitrotoluene Mixture Technical (ratio between 2,4- and 2,6-isomers is about 80:20), (2) Dinitrotoluene Mixture Blend G Technical (isomer ratio 3 to 2), (3) Dinitrotoluene Oil Technical (isomer ratio 1 to 1), (4) Dinitrotoluene Oil 26° Technical (isomer ratio 1 to 1), and (5) 2,4-Dinitrotoluene Technical (high percentage of 2,4-isomer) (See Table 6, p. 20). In contrast, 2,4,6-trinitrotoluene (TNT) must be substantially free from other unsymmetrical isomers because the unsymmetrical isomers explode randomly (Nay, 1972). Frequently, the final product requires more than one synthesis step, and, as a result, the contaminant in the final product may have been formed several steps before the final synthesis step. The similarity between the physical properties of the isomers frequently makes complete purification economically infeasible.

Other contaminants besides related isomers may be found in the final product due to by-product formation, retention of the reagents, or unreacted starting material. For example, nitrobenzoic acids and tetranitromethane may be formed as by-products from the nitration of toluene (Matsuguma, 1967a); some of the mixed acid (sulfuric and nitric) used in nitration steps may be retained, and some iron or sulfur might remain when reduction is the final synthesis step. However, these contaminants are usually found in very low concentration because their physical properties allow easy removal.

Several researchers have analyzed commercial grades of nitroaromatics to determine their purity. Tucker and Schwartz (1971) found that certain defects in oxidation hair colors could be traced to impurities in the chemical intermediate. They analyzed a number of chemical intermediates before and after purification (by recrystallization) (See Table 3), but did not attempt to identify the organic contaminants (However, infrared spectra of the sludges from recrystallization were given).

Venturella *et al*. (1973) developed a gas chromatographic method which they used to determine the purity of crude samples of a variety of nitroaromatic compounds. Their results are listed in Table 4. A similar GC study of raw and crude TNT by Gehring and Shirk (1967) identified the concentrations of impurities (See Table 5).

Table 3. Analysis of Several Nitroaromatic Chemical Intermediates Before and After Recrystallization

(Tucker and Schwartz, 1971)

Compound	Purity (%)	Ash (%)	Iron (ppm)	Melting Point
Nitro-*o*-phenylenediamine				
Raw	95.4	0.20	178	199.4
Purified	99.5	0.025	36	202.5
Nitro-*p*-phenylenediamine				
Raw	90.1	0.20	363	134.0
Purified	98.1	Trace	Trace	143.6
4-Amino-2-nitrophenol				
Raw	95.1	0.82	382	130
Purified	99.4	Trace	Trace	136
2-Amino-5-nitrophenol				
Raw	95.0	0.39	28	186
Purified	98.8	0.02	34	201
2-Amino-4-nitrophenol				
Raw (11.6% water)	75.0	0.06	230	93.0 (hydrate)
Purified	97.9	0.07	69	110.0 (hydrate)

Table 4. Crude Sample Analyses
(Venturella *et al*., 1973)

Compound	% Purity
2,6-Dichloro-4-nitroaniline	86.0
2-Chloro-4,6-dinitroaniline	90.47
2-Bromo-4,6-dinitroaniline	98.33
3-Nitro-4-chloroaniline	93.56
2-Chloro-5-nitroaniline	92.75
2-Nitro-4-chloroaniline	100.3
2,5-Dichloro-4-nitroaniline	87.25
2-Amino-5-nitroanisole	94.65
2-Amino-4-nitroanisole	97.52
3-Nitro-4-aminoanisole	90.93
2-Nitro-5-chloroanisole	98.51

Table 5. Percentage Concentration of Impurities in Typical Samples of Production TNT (2,4,6-Trinitrotoluene)
(Gehring and Shirk, 1967)

	Nitrotoluene Impurities (wt %)					
	2,5-DNT	2,4-DNT	3,5-DNT	2,3,5-TNT	2,4,5-TNT	2,3,4-TNT
Crude TNT	0.02-0.05	0.40-0.81	0.02-0.03	~ 0.02	1.35-2.22	0.80-1.30
Refined TNT	0.02-0.04	0.25-0.40	---	---	0.34-0.45	0.30-0.42

Table 6 provides information on a number of commercial products offered by the Du Pont Company. Both the sales specifications and contaminants are noted; these are typical of the information available on the purity of the commercial nitroaromatics.

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Table 6. Sales Specifications and Contaminants in Technical Grades of Nitroaromatic Compounds

Compound	Specifications	Contaminants	Reference
Nitrobenzene	wt % min. 99.8 distill. range °C max - 0.8 (first drop to 95%, incl. 210.8°C) freezing point, °C min. 5.1 color - yellow	dinitrobenzene, wt% max 0.1% nitrothiophene - none water, wt% max - 0.1% acidity (cacld. as HNO_3), wt% max - 0.001	Du Pont (1974)
o-Nitrotoluene, technical	distill. range °C max - 0.5 (5 - 95%, incl. 222.0°C)	*p*- and *m*-nitrotoluene - 0.5% water, wt% max - 0.2%	Du Pont (1965c)
p-Nitrotoluene, technical	freezing point, °C min. 51.2	*m*-nitrotoluene - 0.3% *o*-nitrotoluene - 0.3% water, wt% - 0.3% acid free	Du Pont (1966c)
2-Chloro-5-nitrobenzene-sulfonic acid, sodium salt, technical	min. purity 40.0% max impurities insoluble in water - 0.15 wt%	water, wt% - 50% sulfuric acid and sodium sulfate - 5% p-chloronitrobenzene - traces	Du Pont (1965b)
2,4-Dinitrotoluene, technical	solid state - light yellow to buff crystals, 90% pass 2 mm screen molten state - crystalline solid below 68.5°C	water, wt% solid - 0.25% molten - none max acid content (as H_2SO_4) - 0.005 wt	Du Pont (1965a)
Dinitrotoluene mixture, technical	2,4 - isomer - 80 ± 1% 2,6 - isomer - 20 ± 1% freezing point, min. - 56.0°C	*o*- or *p*-dinitro isomers - 5% water, wt% - 0.5% acid or alkali - none	Du Pont (1966a)
Dinitrotoluene oil, technical	2,4 - isomer - 50% 2,6 - isomer - 50% freezing point 30.0 ± 5.0°C nitrogen content, min. - 15.15%	acidity, max (as H_2SO_4) - 0.05% water, wt% - 0.5%	Du Pont (1970b)
Dinitrotoluene mixture blend C, technical	2,4 - isomer - 60% 2,6 - isomer - 40% freezing point, max 44.0°C	acidity, max (as H_2SO_4) - 0.05% water, max - 0.5% no odor of *o*-nitrotoluene	Du Pont (1970a)
Dinitrotoluene oil 26°, technical	2,4 - isomer - 50% 2,6 - isomer - 50% freezing point 26.0 ± 3.0°C nitrogen content, min - 15.15%	acidity, max (as H_2SO_4) - 0.05% water, wt% - 0.5%	Du Pont (1970c)
3-Nitro-4-aminoanisole, technical	freezing point, min. 122.8°C purity, min. - 99.0% by wt		Du Pont (1966b)
o-Nitroanisole, technical	light transmittance, min. 50% solution in alcohol at 525 nm - 40% freezing point, min. 10.0°C	*o*-chloronitrobenzene - trace nitrophenols, max - 0.01% by wt water, max - 0.5% by wt	Du Pont (1966f)
p-Nitroanisole, technical	freezing point, min. 51.0°C	*p*-nitrophenol trace chloronitrobenzene - trace water, max - 0.5% by wt	Du Pont (1966e)
p-Nitrobenzoic acid, technical	purity, min. - 99.5% min. light transmission of 6.3% solution in aqueous alcohol - NaOH - 75%	*p*-nitrotoluene - trace mineral acids - trace water, max - 0.25%	Du Pont (1971b)
o-Nitrochlorobenzene, technical	freezing point, min. 31.8°C matter insoluble in alcohol, max - 0.2%	*m*- or *p*-chloronitrobenzene - 1% *m*- or *p*-chloronitrobenzene - trace water, max - 0.1%	Du Pont (1971c)
p-Nitrochlorobenzene, technical	purity - 99.5% freezing point, min. 82.5°C	*o*-chloronitrobenzene - trace water, max - 0.15%	Du Pont (1966g)
p-Nitrophenol, technical	purity, min. - 99.5% by wt melting point 111 - 114°C	water - 1.0% ash, max - 0.10% iron, max - 25 ppm	Du Pont (1971a)
p-Nitro Sodium Phenolate, technical	purity, min. - 76.0% by wt optical density of chloroform extract at 400 nm - 0.10% material insoluble in hot water - 0.05%	sodium chloride *p*-chloronitrobenzene - trace	Du Pont (1966d)

B. Chemistry

1. Reactions Involved in Uses

Historically, the first commercial uses of nitroaromatic compounds were as chemical intermediates for the dye and pigment industry. Much of the chemistry was developed in the late 1800's (e.g., Aniline was first commercially manufactured from nitrobenzene in 1874; Kouris and Northcott, 1963). Presently, the chemical intermediate uses of nitroaromatics for rubber chemicals, photographic chemicals, and drugs are similar in commercial importance to the applications in dyes and pigments. The thermal instability imparted by multiple nitro group substitution is also important to the use of polynitroaromatic compounds as explosives.

Explosives are materials "that can undergo very rapid self-propagating decomposition or reaction of ingredients with the consequent formation of more stable materials, the liberation of heat, and the development of a sudden pressure through the action of its heat on produced or adjacent gases" (Rinkenbach, 1965). The chemistry of the decomposition process is not well understood, but it appears to be closely related to the high oxygen content of compounds containing several nitro substituents. Rinkenbach (1965) indicates that the oxygen balance (oxygen content relative to the total oxygen required for complete oxidation) is a very important property of most explosives. Maksimov (1972) concluded that the autocatalytic decomposition of gaseous nitroaromatic compounds at 270-350°C was quite dependent upon the substituents on the ring. He found that the tendency for decomposition increases with the number of nitro groups and is accelerated by the presence of hydroxyl, methyl, bromo, fluoro, chloro, and amino substituents. The amino and chloro derivatives were the most stable, while the stability of nitrobenzenes decreases in the order: m-dinitrobenzene > p-dinitrobenzene > o-dinitrobenzene > 1,2,3-trinitrobenzene > 1,3,5-trinitrobenzene.

Substitution of a nitro group on an aromatic ring may have several synthetic functions. Perhaps the most important use of nitro substitution is as a starting point for the preparation of amines (Bannister and Olin, 1965). There are two major processes that can be used to prepare amines: (1) amination by ammonolysis and (2) amination by reduction of nitro groups (Shreve, 1963). The commercial approach selected depends upon several factors, such as the other ring substituents and process economics. The production of aniline by vapor phase reduction of nitrobenzene (about 97% of the nitrobenzene produced is consumed in this way) is typical of reductive amination.

Actually, reduction of nitro functional groups that are attached to aromatic rings can result in a variety of products, depending upon the reaction conditions used (see Figure 2). Reduction can be accomplished by employing any of the following reagents (Shreve, 1963).

(1) Hydrogen or carbon monoxide in the presence of a catalyst, in liquid or vapor phase.

(2) Iron in acid or neutral solutions (tin or zinc occasionally used).

(3) Zinc or iron in alkaline solution.

(4) Sulfides in alkaline solution.

(5) Miscellaneous reducing agents

(a) Sodium hydrosulfite, $Na_2S_2O_4$, in alkaline solution.

(b) Sodium sulfite, Na_2SO_3, in solution.

(c) Electrolytic action.

(d) **Metal hydrides.**

The old Bechamp process, which used iron turnings and acid, is gradually being replaced by more efficient catalytic hydrogenation in large-scale manufacturing process (e.g.,production of aniline, toluidines, xylidines, phenylenediamines, and toluenediamines).

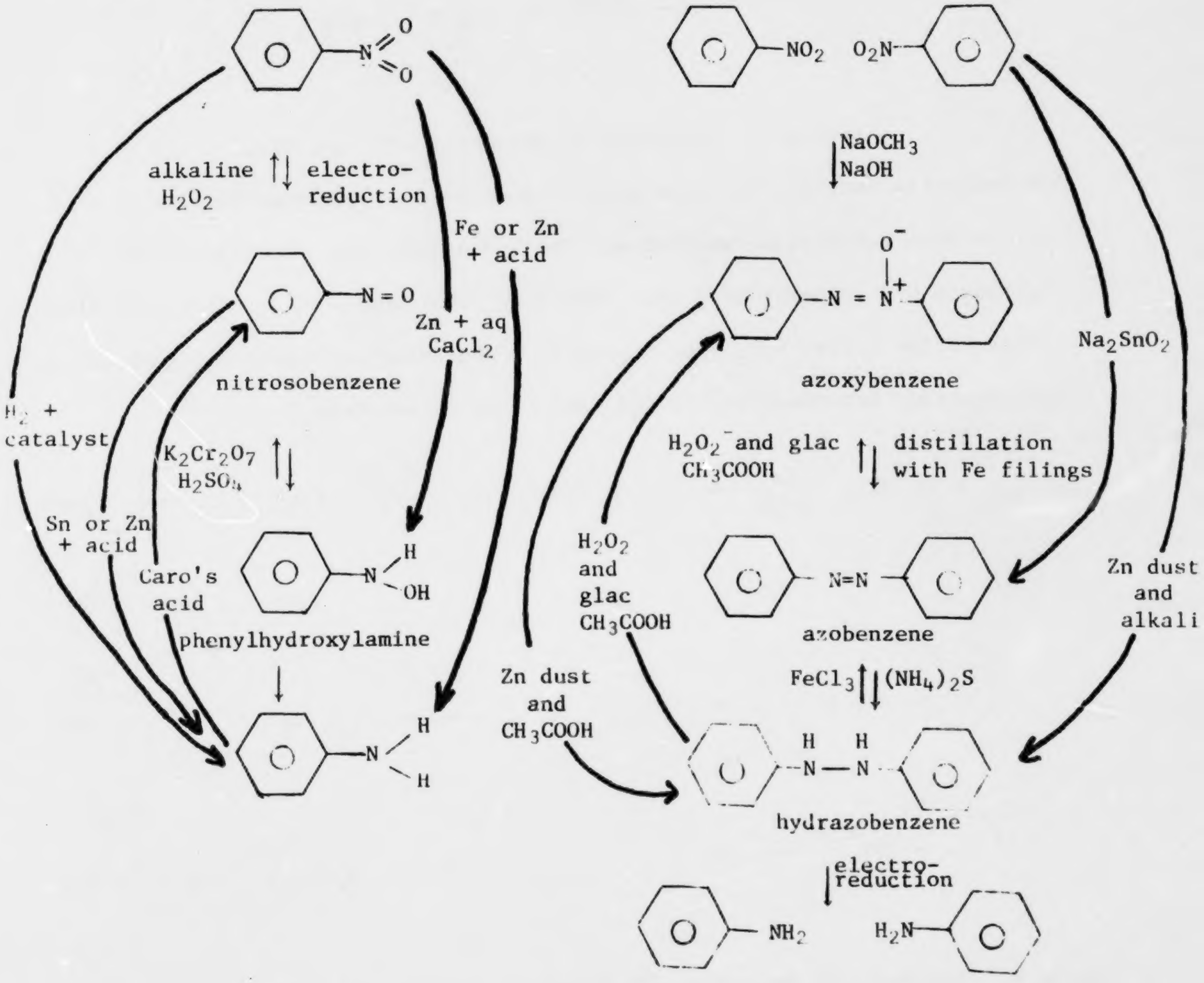


Figure 2. Reduction Products of Nitroaromatic Compounds (Shreve, 1963)

As can be seen in Figure 3, reduction under alkaline conditions usually results in some form of dimerization. The reduction of nitrobenzene with zinc dust under acid, neutral, and basic conditions illustrates the variety of products that can be formed.

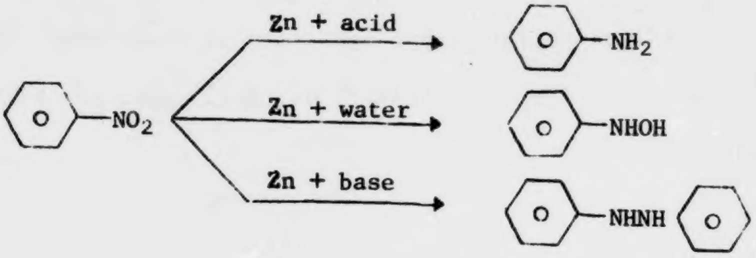


Figure 3. Reduction of Nitrobenzene Using Zinc

The reductive coupling of nitro groups under basic conditions happens to have considerable commercial importance. The hydrazobenzenes that are formed can be rearranged by treatment with acid (benzidine rearrangement) to form benzidine and benzidine derivatives (see Figure 4). Benzidine and benzidine derivatives are important intermediates in the production of azo dyes.

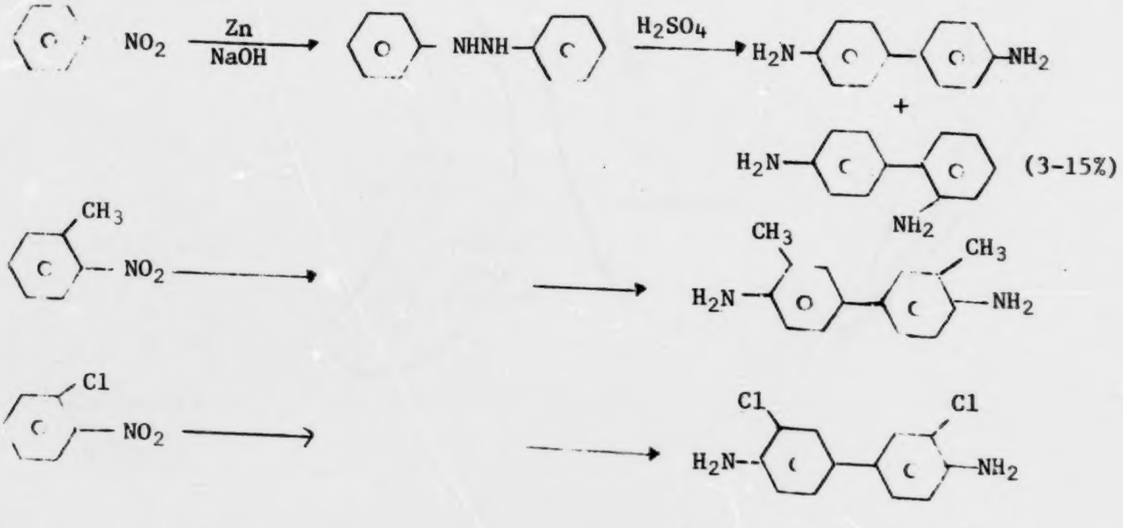


Figure 4. Synthesis of Benzidine and Benzidine Derivatives from Nitroaromatics (Lurie, 1964).

Nitro groups are usually introduced into aromatic systems by direct nitration with mixed sulfuric and nitric acids. The process is considered to be an electrophilic attack by the nitronium ion, $+NO_2$. Because nitro groups inductively withdraw electrons out of the aromatic ring, they deactivate the ring to further electrophilic substitution; therefore, subsequent nitration, or other electrophilic substitution (e.g., chlorine, Friedel-Crafts alkylation, etc.), is much more difficult. The nitro group is also a *meta* director for electrophilic substitution, due to the resonance structures depicted in Figure 5 (the relatively negative *meta*-position is more susceptible to electrophilic attack).

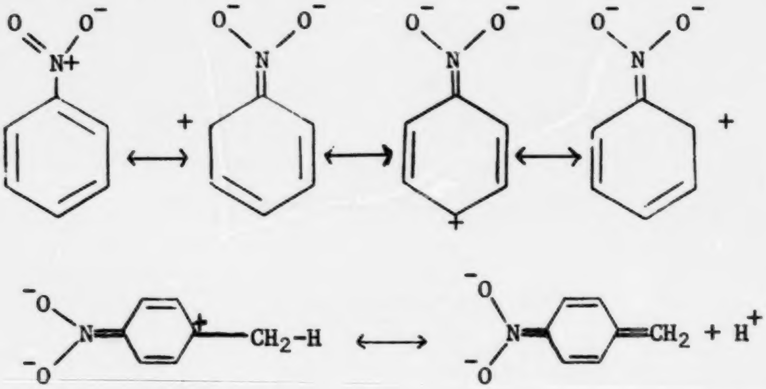


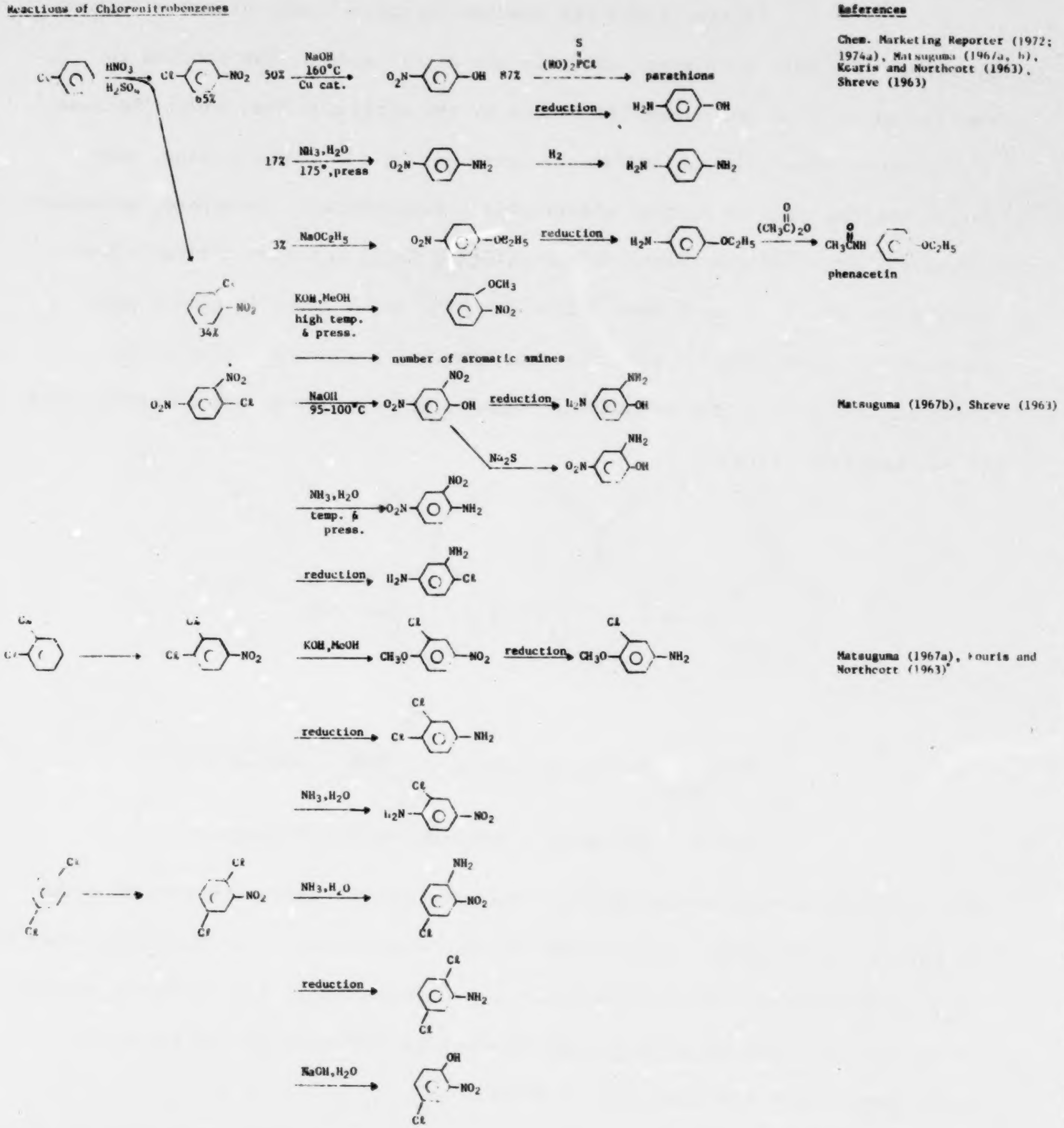
Figure 5. Resonance Structures of Nitroaromatics

The resonance structures in Figure 5 also explain the susceptibility of *ortho*- or *para*-, but not *meta*-, substituted chloronitrobenzenes to nucleophilic substitution (e.g.,*p*-chloronitrobenzene hydrolysis to *p*-nitrophenol). The increased acidity of methyl hydrogens or *o*- or *p*-nitrotoluenes is explained by the resonance structures in the lower portion of Figure 5.

The commercial use of nitration and the use of the resulting nitroaromatics as chemical intermediates are illustrated in Figure 6. In order to understand the logic behind the synthetic approaches, the resonance structures discussed above, as well as the directing affects of various substituents, must be considered (e.g. $-CH_3$,$-OH$,$-Cl$,$-Br$,$-OR$,$-NH\overset{O}{\overset{\|}{C}}R$ are *ortho*-*para* directors, while

Reactions of Chloronitrobenzenes

References



Chem. Marketing Reporter (1972; 1974a), Matsuguma (1967a, b), Kouris and Northcott (1963), Shreve (1963)

Matsuguma (1967b), Shreve (1963)

Matsuguma (1967a), Kouris and Northcott (1963)

Figure 6. Commercial Chemistry of Nitroaromatic Compounds

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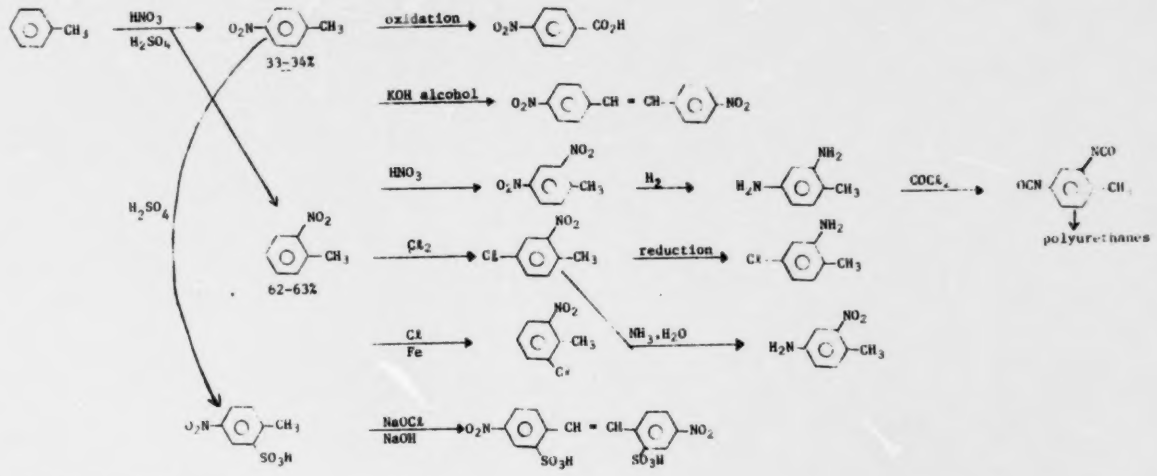
Reactions of Nitrotoluenes

Mutsuguma (1967a), Shreve (1963), Schwander and Dominguez (1969)



Formation of Aromatic Amines

Chemical Marketing Reporter (1974b), Shreve (1963), Thirtle (1968)

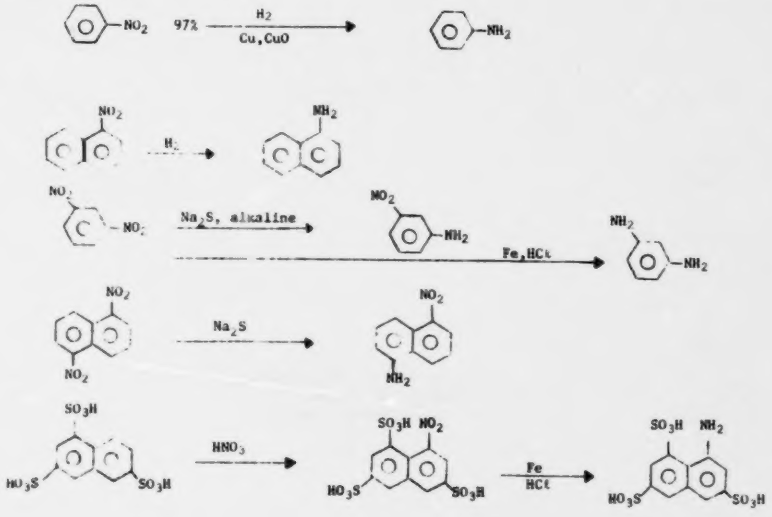
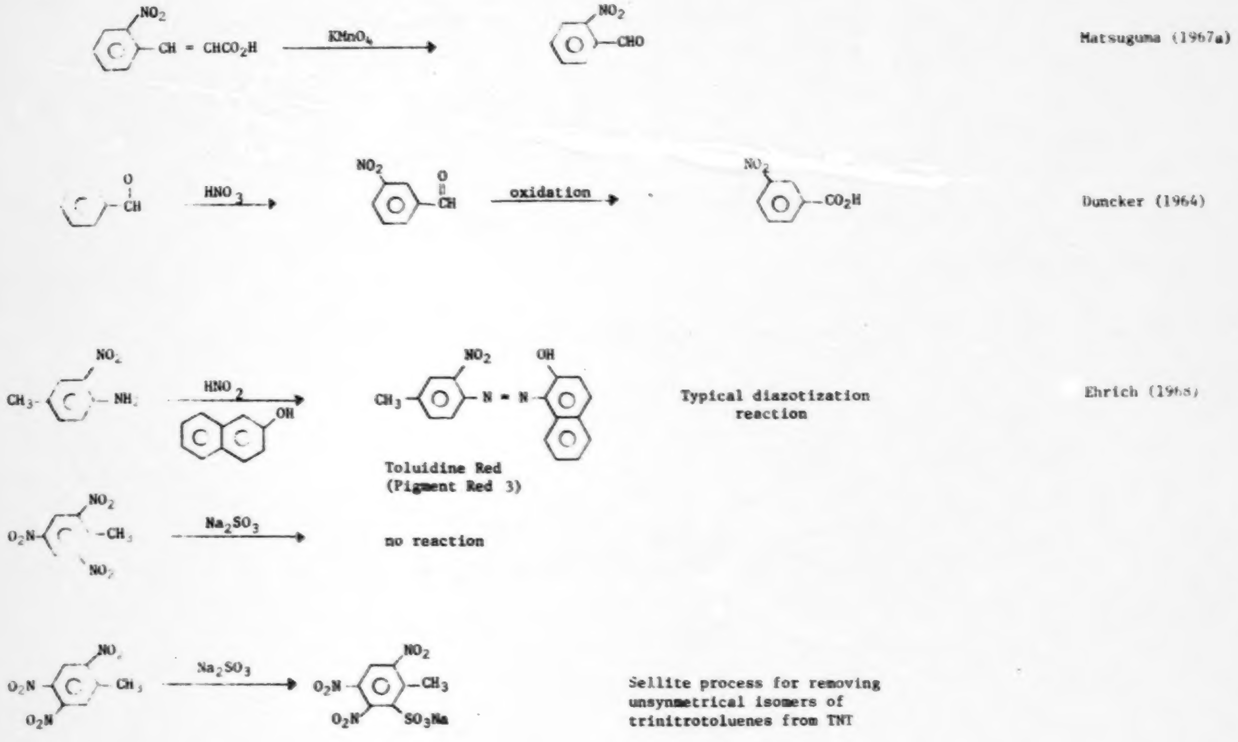


Figure 6. Commercial Chemistry of Nitroaromatic Compounds (Cont'd)

Miscellaneous



Matsuguma (1967a)

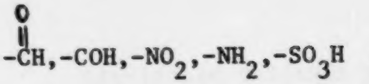
Duncker (1964)

Typical diazotization reaction

Ehrich (1968)

Sellite process for removing unsymmetrical isomers of trinitrotoluenes from TNT

Figure 6. Commercial Chemistry of Nitroaromatic Compounds (Cont'd)



$-\overset{\overset{\displaystyle O}{\|}}{C}H, -COH, -NO_2, -NH_2, -SO_3H$ are meta directors). Also important in determining the appropriate synthetic approach is the fact that nitric acid used as a nitrating reagent is also capable of oxidizing some of the starting material. As a result, poor yields preclude the commercial use of direct nitration of chemicals such as phenol and benzoic acid. For this reason, chloronitrobenzenes are used to synthesize nitrophenols and aminophenols.

2. Hydrolysis

Nitroaromatics are generally very stable in water under neutral conditioning. Hoffsommer and Rosen (1973) examined the hydrolysis of 2,4,6-trinitrotoluene (TNT) and N-methyl-N-nitro-2,4,6-trinitroaniline (tetryl) in sea water. For several months, they stored approximately saturated solutions (TNT = 95 ppm; tetryl = 26 ppm) of the compounds in sea water (pH = 8.1). After 101 days, only 12% of the tetryl remained, as measured by gas chromatography with an electron capture detector (GC-EC). The ultraviolet spectrum of the aqueous solution suggests the formation of picric acid (2,4,6-trinitrophenol), an expected hydrolysis product. With TNT, no change in concentration was observed by GC-EC measurement after 108 days at room temperature. The nucleophilic reactivity of positions ortho or para to nitro substituents has been discussed in Section I-B-1 (see Figure 5, p. 25). In fact, this hydrolytic reactivity is utilized in the synthesis of nitrophenols and nitroethers from chloronitrobenzene. The difference in the reactivities of tetryl and TNT in sea water can be attributed to the difference in leaving groups; the methyl anion ($^{-}CH_3$) in trinitrotoluene makes a poor leaving group compared to the substituted amino group in tetryl.

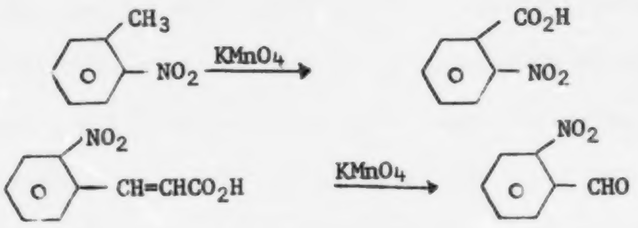
Aromatic nucleophilic substitution at positions ortho or para to nitro groups is well known (Murto and Murto, 1966; Murto, 1966). The neutral hydrolysis rate constants for some 2,4,6-trinitrobenzene derivatives are fast enough to be measured. In contrast, only the alkaline hydrolysis rate constants of 2,4-dinitrobenzene derivatives can be measured. The kinetic data developed by Murto (1966) and Murto and Murto (1966) are presented in Table 7. Both the neutral and basic hydrolysis reactions probably proceed by an S_N2 mechanism. The kinetic data demonstrated that the reaction is dependent upon the hydroxyl ion concentration (i.e.,it is pH dependent) and the concentration of the nitroaromatic compound. The effect of pH can be demonstrated with 2,4-dinitrochlorobenzene: at pH 13.3, the measured half-life at 25°C is 3800 seconds; at neutral conditions (pH 7.0), the calculated half-life is 1 x 10^7 hours. Elevated temperatures may affect the reaction rate significantly. For example, Urbanksi (1964) notes that when 2,4-dinitrochlorobenzene and 2,4,6-trinitrochlorobenzene are boiled in water for 5 hours, the percent that hydrolyzes is ~1% and 26.6%, respectively.

Table 7. Alkaline and Neutral Hydrolysis Rates of Nitroaromatic Compounds in Water (Murto, 1966; Murto and Murto, 1966)

Compound	Initial NaOH Concentration (N)	Initial pH	k (ℓ mole^{-1}s^{-1}) 25°C	$t_{1/2}$ (sec)
$2,4,6-(NO_2)_3C_6H_2OCH_3$	0.005	12.3	1.28	0.5
$2,4,6-(NO_2)_3C_6H_2OC_6H_5$	0.005	12.3	1.56	0.4
$2,4,6-(NO_2)_3C_6H_2F$	0.005	12.3	700	0.001
$2,4,6-(NO_2)_3C_6H_2C\ell$	0.005	12.3	0.506	1.4
$2,4,6-(NO_2)_3C_6H_2OCH_3$	-	7.0	0.199×10^{-5}	3.5×10^5
$2,4,6-(NO_2)_3C_6H_2OC_6H_5$	-	7.0	0.144×10^{-5}	4.8×10^5
$2,4,6-(NO_2)_3C_6H_2NO_2$	-	7.0	75.7×10^{-5}	9.2×10^2
$2,4,6-(NO_2)_3C_6H_2F$	-	7.0	144×10^{-5}	4.8×10^2
$2,4,6-(NO_2)_3C_6H_2C\ell$	-	7.0	0.00644×10^{-5}	1.1×10^7
$2,4-(NO_2)_2C_6H_3OCH_3$	0.05	13.3	34×10^{-5}	2.0×10^3
$2,4-(NO_2)_2C_6H_3OC_6H_5$	0.05	13.3	20×10^{-5}	3.5×10^3
$2,4-(NO_2)_2C_6H_3NO_2$	0.05	13.3	8520×10^{-5}	8.2
$2,4-(NO_2)_2C_6H_3F$	0.05	13.3	12900×10^{-5}	5.4
$2,4-(NO_2)_2C_6H_3C\ell$	0.05	13.3	185×10^{-5}	3.8×10^3

3. Oxidation

Nitro groups are already in a high state of oxidation and, therefore, have little susceptibility to oxidation conditions. In fact, nitrobenzene has been used as a mild oxidizing agent (Matsuguma, 1967a). The oxidative stability of the nitro group, as well as the instability of other functional groups, is illustrated by the following equations:



4. Photochemistry

In order for a compound to react photochemically, it has to be able to derive energy from the available light and then convert the energy into some type of chemical transformation. Photochemical processes are extremely complex and are difficult to predict; they are dependent upon such parameters as the ultraviolet absorption spectrum and quantum yields (efficiency of reaction based on the quantity of incident light absorbed) of the chemicals or available sensitizers, and the reaction medium in which the chemical is irradiated (this affects the previous parameters as well as the reaction that the excited state may undergo). As a result, environmental photochemistry is extremely difficult to simulate because the conditions can vary so much. However, there is fairly good agreement that the incident light (sunlight) contains only wavelengths longer than 290 nm because the ozone in the stratosphere effectively filters out the shorter wavelengths (higher energy light) (Koller, 1965).

Substitution of a nitro functional group on an aromatic system has a distinctive bathochronic effect upon its ultraviolet spectrum. In fact, nitrobenzene (λmax = 268.5, εmax = 7800) shows the greatest red shift (toward longer wavelengths) of all the common monosubstituted benzenes (Jaffe and Orchin, 1962) (for comparison benzene λmax = 203.5, εmax = 7400). This has been attributed to the particularly strong resonance interaction between the nitro group and the aromatic ring. This resonance interaction and, consequently, the ultraviolet absorption spectrum, is affected by other substitutents and by their position on the aromatic ring. This is illustrated for a number of nitroaromatic compounds in Table 8. The λmax for the ultraviolet absorption spectrum of the majority of the compounds considered for this review are listed in Table 1. The reported λmax in Tables 1 and 8 are only for the primary absorption band (^1L_a band, $\pi \longrightarrow \pi^*$ transition). Frequently, there is a much less intensive band located at longer wavelength (^1L_b band, $n \longrightarrow \pi^*$ transition) which is capable of absorbing sunlight wavelengths (see Table 10 for examples). With nitrobenzene, the ^1L_b band is considered to be under the ^1L_a band.

The ultraviolet absorption spectrum of any given nitroaromatic compound can be considerably affected by the medium or solvent in which the spectrum is measured. This is generally true for aromatic systems with electron-accepting substituents. This shift of the UV absorption spectra is illustrated for nitrobenzene in Table 9 and for a number of compounds in Table 8.

Table 8. Ultraviolet Spectra of Representative Nitroaromatic Compounds (Sandus and Slagg, 1972; Hashimoto and Kano, 1972; Jaffe and Orchin, 1962)

Compound	Solvent	L_a band λmax, nm	εmax, liter mole^{-1} cm^{-1}
Nitrobenzene	*n*-heptane	252	8810
Nitrobenzene	isopropyl alcohol	258	8500
o-Nitrotoluene	*n*-heptane	251	5890
m-Nitrotoluene	*n*-heptane	256.5	8190
p-Nitrotoluene	*n*-heptane	264	10100
m-Dinitrobenzene	*n*-heptane	228	18800
2,4-Dinitrotoluene	*n*-heptane	233	15800
2,4,6-trinitrotoluene	cyclohexane	224.5	23000
p-Nitrobenzonitrile	isopropyl alcohol	256	1330
Ethyl *p*-Nitrobenzoate	isopropyl alcohol	257	1340
Isopropyl *p*-Nitrobenzoate	isopropyl alcohol	256	1290
p-Nitrobenzoic acid	isopropyl alcohol	255	1200
p-Nitrotoluene	isopropyl alcohol	270	1050
p-Nitroanisole	isopropyl	300	1110
p-Chloronitrobenzene	water	280	
p-Nitrophenol	water	317	
p-Nitroaniline	water	381	
p-Nitroaniline	cyclohexane	323	
p-Nitrophenol	cyclohexane	286	

Table 9. Solvent Effects on Nitrobenzene Ultraviolet Spectra

Solvent	Water	Ethanol	Heptane	Vapor
λ_{max}, nm	265.5	259.5	251.8	239.1

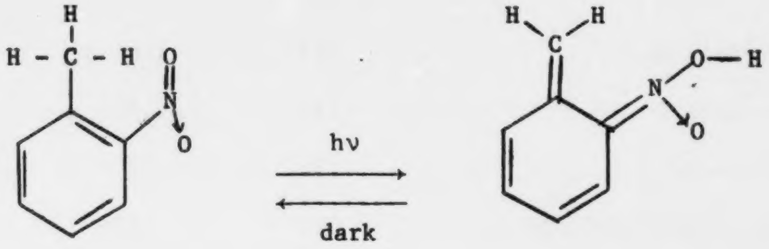
A similar effect may occur for nitroaromatic compounds in the absorbed state, a condition that frequently takes place in the environment and can alter the energy required to bring about photodecomposition (Plimmer, 1972). The absorption spectrum shifts result from perturbations of the electronic states of the molecules upon adsorption. Robin and Trueblood (1957) observed such absorption spectra changes for several nitroaromatic compounds after adsorbing the compounds on silicic acid in cyclohexane solution. Their results are presented in Table 10. In a similar study of pesticides adsorbed on silica gel, Plimmer (1972) noted that trifluralin (α, α, α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) exhibited the largest red shift (60 nm) of all the compounds tested. Keeping in mind the many factors mentioned above that may affect the propensity of nitroaromatic compounds to photochemically react, the available studies on photochemistry will be reviewed in the following paragraphs.

A number of researchers have studied the fundamental and mechanistic aspects of nitroaromatic photochemistry. The relevance of these studies to environmental conditions is unknown, but they do provide some insight into the process. Frequently, these studies use light sources that contain wavelengths of higher energy than those found in sunlight (254 nm light is used frequently).

Table 10. Ultraviolet Absorption Spectrum Changes Caused by Adsorption on Silicic Acid (Robin and Trueblood, 1957)

Compound	λ (nm) Cyclohexane (C_6H_{12})	λ (nm) Silicic Acid (SA) and Cyclohexane	$\varepsilon_{C_6H_{12}}/\varepsilon_{SA}$
2-Nitroaniline	375-378	413	
	270	285	
	246	240	
	229		
4-Nitroaniline	323	374-382	3.6
4-Nitroanisole	294	316	1.2
Nitrobenzene	253	271	1.3
4-Nitro-N-ethylaniline	344	420	
	∿277		
4-Nitro-N,N-diethylaniline	360-366	420	
	234		
2-Nitrophenol	345-349	352-356	
	272	284-286	
4-Nitrophenol	286	315	1.3

Several investigators have used high energy light to study the photochromism (formation of colored species upon exposure to light) of *o*-alkylnitrobenzenes which contain at least one benzylic hydrogen. Using a flash photolysis technique with light containing wavelengths greater than 250 nm, Wettermark (1962a,b) and Wettermark and Ricci (1963) were able to detect and measure the disappearance rate constant of a colored isomer in an aqueous solution (λ_{max} of the colored isomer was 360-410 nm, depending upon the pH) of *o*-nitrotoluene and 2,4-dinitrotoluene. They suggested the mechanism depicted in Figure 7.



Nitro Form

Aci Form

Figure 7. Suggested Mechanism to Explain Photochromism of *o*-Alkylnitrobenzenes

Using deuterium oxide and *o*-nitrotoluene, Morrison and Migdalof (1965) were able to show that deuterium was incorporated into the methyl group even when >290 nm light (Pyrex filter) was used. They concluded that hydrogen abstraction was definitely taking place. From this data, it is possible to conclude that *o*-alkylnitroaromatic compounds are probably quite susceptible to photochemical alteration, since they isomerize to highly colored compounds which may react further. (For an example of this, see the following discussion of work by Burlinson *et al.*, 1973.)

Of course, many nitroaromatic compounds do not have an abstractable ortho hydrogen. Numerous conditions have been used in studies of these compounds. A favorite solvent is isopropyl alcohol, which contains the abstractable hydrogen missing in non-o-alkylnitrobenzenes. Hurley and Testa (1966, 1967) photolyzed nitrobenzene in isopropanol with 366 nm light. They concluded that phenylhydroxylamine is generally the initial product, but an oxidation in air to nitrosobenzene, which then couples with the hydroxylamine to form azoxybenzene, also takes place. The measured quantum yield was approximately 10^{-2}, and the overall photochemical reduction involved four hydrogen abstractions. Hashimoto and coworkers (1968) found similar results using 330-340 nm light. However, they found that, by carrying out the reaction in the presence of hydrochloric acid and a sensitizer, the final reduction products were aniline, p-aminophenol, and p-chloroaniline.

Hashimoto and Kano (1970, 1972) have studied the photochemical reduction of substituted nitrobenzenes in isopropanol under nitrogen atmosphere using 316 and 366 nm light. They found that benzenes with electron-withdrawing substituents yielded aniline derivatives, while the use of benzenes with electron-donating substituents resulted in the formulation of the hydroxylamine derivative (see Table 11). They also found a quantitative correlation between the quantum yield and the Hammett constant. When the experiments were run in air-saturated isopropanol using 313 nm light, the photochemical reaction was completely quenched. This result suggests that photoreduction of nitroaromatics proceeds by an excited triplet state. Most of the photolysis experiments were conducted in isopropanol. However, with p-nitrobenzonitrile, other solvents were used and a considerable solvent effect on the quantum yield was noted (Table 12).

Table 11. Quantum Yield and Products from the Disappearance of Substituted Nitrobenzenes in 2-Propanol Under Nitrogen Atmosphere (Hashimoto and Kano, 1972)

$XC_6H_4NO_2$	Conc. x 10^3 mol/1	Light nm	Product	Quantum Yield
p-NO_2	1.00	366	p-Nitroaniline	0.16
p-CN	1.03	313	p-Aminobenzonitrile	0.48*
m-CN	1.00	313	m-Aminobenzonitrile	0.34
p-$COOC_2H_5$	1.00	313	Ethyl p-aminobenzoate	0.15
m-$COOC_2H_5$	1.00	313	Ethyl m-aminobenzoate	0.11*
p-$COOCH(CH_3)_2$	0.25	313	Isopropyl p-aminobenzoate	0.15
p-COOH	1.00	313	p-Aminobenzoic acid	0.12
m-COOH	1.17	313	m-Aminobenzoic acid	0.18
H	1.10	313	Phenylhydroxylamine	0.03
p-CH_3	1.00	313	p-Hydroxylaminotoluene	0.07
p-OCH_3	0.25	313	p-Hydroxylaminoanisole	0.02
p-OH	0.50	313	---	0.00
p-NH_2	0.50	366	---	0.004

* Quantum yield for formation of Anilines.

Table 12. Solvent Effect for Photoreduction of p-Nitrobenzonitrile (Hashimoto and Kano, 1972)

Conc. (10^3 mol/l)	Solvent	Quantum Yield
1.03	Isopropanol	0.48
1.00	Ethanol	0.11
1.00	Cyclohexanane	0.00

Letsinger and McCain (1969) and Wubbels and Letsinger (1974) have photolyzed nitroaromatic compounds in aqueous solutions which contained high anion concentrations. Letsinger and McCain (1969) photolyzed (light >290 nm) 4-nitroanisole (10^{-4}M) in an aqueous solution of potassium cyanide (4×10^{-3}M) that had been well purged with nitrogen. They isolated 2-cyano-4-nitroanisole and 3,3'dicyano-4,4'-dimethoxyazobenzene. Under similar light conditions but in 12N hydrochloric acid solution, Wubbels and Letsinger (1974) found that the nitro group of various nitroaromatics was usually reduced to an amine, and the ring was substituted with one or more chlorine atoms.

Barltrop and Bunce (1968) studied the photoreduction of nitroaromatic compounds under a variety of conditions and light sources. One of the more interesting studies is the photoreduction of nitrobenzene with >290 nm light in various solvents (see Table 13). Since the presence of oxygen was shown to have little effect, the experiments in Table 13 were run without deoxygenation. In solvents having readily abstractable hydrogen atoms, no

Table 13. Solvent Dependence of the Products of the Photoreduction of Nitrobenzene with Light $\lambda > 290$ nm (Barltrop and Bunce, 1968)

Solvent	Irradiation time (hr.)	Conversion (%)	Aniline	Azobenzene	2-Hydroxyazobenzene
Petroleum	5	26	4	<2	<2
Toluene	5	64	<1	--	--
Ether	5	72	44	2	1
Isopropyl alcohol	5	86	13	--	--
Isopropyl alcohol	3	60	19	--	--
_t_-Butyl alcohol	5	39	--	--	--
50% Toluene/_t_-butyl alcohol	5	23	--	--	--
Diethylamine	5	76	6	20	31
Triethylamine	5	97	14	12	5
33% Aq./diethylamine	2	84	5	8	17
50% Aq./isopropyl alcohol	3	53	40	--	--
50% Ammonia/isopropyl alcohol	2	58	16	4	7

In all cases nitrobenzene (0.5 g) was irradiated in 100 ml. of solvent; percentage yields are based on nitrobenzene consumed. The absence of a column entry means that this compound could not be detected.

azoxy compounds were detected, although these products have been noted by other authors. Sandus and Slagg (1972) have suggested that the poor reproducibility might be attributable to photolysis of the intermediates (e.g., nitroso derivatives), which could take place with 310-360 nm light. Because of this suspected photosensitivity of the products, Sandus and Slagg (1972) decided to use 254 nm light for their flash and continuous photolysis studies of nitroaromatics. They concluded that the aci and radical intermediates that have been observed are probably due to side reactions and are not related directly to the products.

A number of nitroaromatic compounds used as pesticides have been tested for photochemical stability. Often the experimental conditions used attempt to simulate conditions that the pesticide may be exposed to in nature, but this has not always been the case. In one of the earliest pesticide photochemistry studies, 254 nm light was used to screen the effect of light on 141 pesticides (Mitchell, 1961) adsorbed on paper (chromatographed after irradiation). The author found that the dinitrophenol pesticides (2,4-dinitro-6-sec-amylphenol, 4,6-dinitro-o-sec-butylphenol, 4,6-dinitro-o-cresol, and 4,6-dinitro-o-cyclohexylphenol) showed little or no degradation, while pentachloro- and tetrachloronitrobenzenes were almost completely degraded. The conditions and results of some other pesticide photolysis studies have been summarized in Table 14. These nitroaromatic pesticides have very unusual chemical structures compared to some of the other commercially important nitroaromatic compounds and, therefore, extrapolation to simpler structures is difficult (e.g., 2,6-dinitroaniline herbicides absorb strongly at 350-450 nm). However, the last two studies are important because they demonstrate that nitro groups can be converted to nitroso or amino functional groups under simulated environmental conditions. The observation that azoxybenzene derivatives can be

Table 14. Photolysis Studies of Nitroaromatic Pesticides

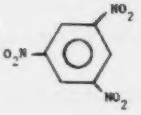
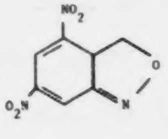
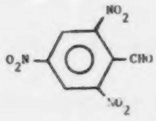
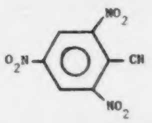
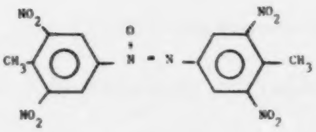
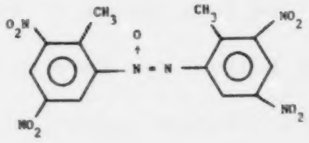
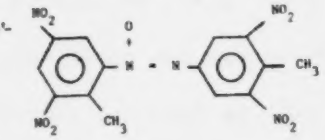
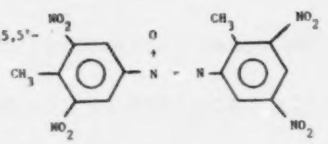
Reference	Compound(s) Studied	Light and Wavelength	Reaction Media	Results
Wright and Warren (1965)	Trifluralin (α, α, α-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine)	Sunlight and artificial light (> 290 nm)	Coated on glass or soil	After 6 hours irradiation on glass, significant change in the UV absorption spectrum had occurred. Degradation on soil very slow.
Hamadmad (1967)	Pentachloronitrobenzene (PCNB)	Sunlight and artificial light (254 nm)	In solution, on TLC plates or on soil	PCNB degraded with 254 nm light but would not decompose with > 270 nm light. With sunlight no effect.
Matsuo and Casida (1970) Bandal and Casida (1972)	2-*sec*-Butyl-4,6-dinitrophenol	Sunlight	Bean leaves	6-Amino-2-*sec*-butyl-4-nitrophenol and other unknowns were formed when sensitizers were present. Isopropyl carbonate derivative is stable.
Parochetti and Hein (1973)	Trifluralin	Sunlight and artificial light (> 290 nm)	Adsorbed on soil	No significant loss from photodecomposition
Brewer *et al*. (1974)	Fenitrothion (0,0-dimethyl-0-(3-methyl-4-nitrophenol) phosphorothioate	Artificial light (> 290 nm)	Vapor phase and in ethanol	4-Nitro-3-methylphenol is the major product. Extended photolysis degraded initial photoproducts. Photolysis of the vapor for 240 hours resulted in ∿ 1-2% decomposition.
Nakagawa and Crosby (1974)	Nitrofen (2,4-dichlorophenyl *p*-nitrophenyl ether) and derivatives (e.g. *p*-nitrophenol)	Sunlight and simulated sunlight	Aqueous suspension 100-200 mg/l of deionized water	Isolated 4-nitrocatechol, 2,4-dichlorophenyl *p*-aminophenyl ether, and 4,4'-bis(2,4-dichlorophenoxy) azobenzene. *p*-Nitrophenol is more photochemically stable than 2,4-dichlorophenol. It degraded to 4-nitrocatechol, hydroquinone, and a nonvolatile, dark polymer.
Plimmer and Klingebiel (1974)	N-*sec*-Butyl-4-*tert*-butyl-2,6-dinitroaniline	Sunlight and artificial light (> 290 nm)	Water solution and methanol TLC silica plates	Decomposed more slowly than trifluralin. Major product in methanol or water was 4-*tert*-butyl-2-nitro-6-nitrosoaniline. Photodecomposition occurred upon irradiation of the TLC plates with sunlight or a sunlamp.

formed is also quite significant, although the rate of such intramolecular reactions would probably be very slow at trace concentrations.

Perhaps the most significant environmental photolysis study of a non-pesticide is the work on the aqueous photodecomposition of trinitrotoluene (TNT) by Burlinson et al. (1973). These authors photolyzed saturated aqueous solutions (120-130 ppm) of TNT with Pyrex-filtered (>290 nm) ultraviolet light to understand why water effluents containing TNT turned pink. (These effluents are commonly referred to as "pink waters.") From a continuous photoreactor where 65% of the TNT had decomposed, the authors were able to extract 20% of the degradation products by benzene extraction. The products isolated and their yields are listed in Table 15. The azoxy derivatives were isolated from a static run (30 hours of sunlight with 120 ppm) where 75% of the TNT had decomposed. Burlinson and coworkers (1973) were unable to isolate and identify any of the 80% of the degradation products that were water-soluble.

Carrying out the TNT photolysis in hydrogen-donor solvents resulted in a 47% yield of the four isomeric tetranitroazoxytoluenes. This illustrates the effect that solvent can have on a photochemical process. With dioxane (d_8) solvent (a good hydrogen donor), they observed no deuterium exchange into the recovered TNT. Also, an inverse relationship between photodecomposition and deuterium uptake was noted when D_2O was used as a solvent. From this information, as well as from the fact that the photodegradation rate is pH dependent, the authors concluded that the aci form was probably not involved in the formation of the azoxy derivatives and that the anion (II) in Figure 8 may be involved in the formation of the major products of aqueous TNT photolysis.

Table 15. Photolysis Products from an Aqueous Solution of TNT (Burlinson *et al.*, 1973)

Compound	Structure	Yield (%)
1,3,5-Trinitrobenzene		0.5-1.0
4,6-Dinitroanthranil		3-4
2,4,6-Trinitrobenzaldehyde		8-10
2,4,6-Trinitrobenzonitrile		3-4
2,2',6,6'-Tetranitro-4,4'-azoxytoluene	 NO_2 O N = N NO_2 CH_3 CH_3 NO_2 NO_2	<1*
4,4',6,6'-Tetranitro-2,2'-azoxytoluene	 O_2N CH_3 O CH_3 NO_2 N = N NO_2 NO_2	<1*
2',4-Dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene	 NO_2 O NO_2 N = N CH_3 NO_2 CH_3 NO_2	<1*
2,4'-Dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene	 NO_2 O CH_3 NO_2 CH_3 N = N NO_2 NO_2	<1*

* Found in only trace amounts in "pink water."

Burlinson et al. (1973) also noted a significant difference between the reactivities of trinitrobenzene and TNT. Photolysis of 1,3,5-trinitrobenzene in aqueous solution produced no photoproducts after six hours of irradiation.

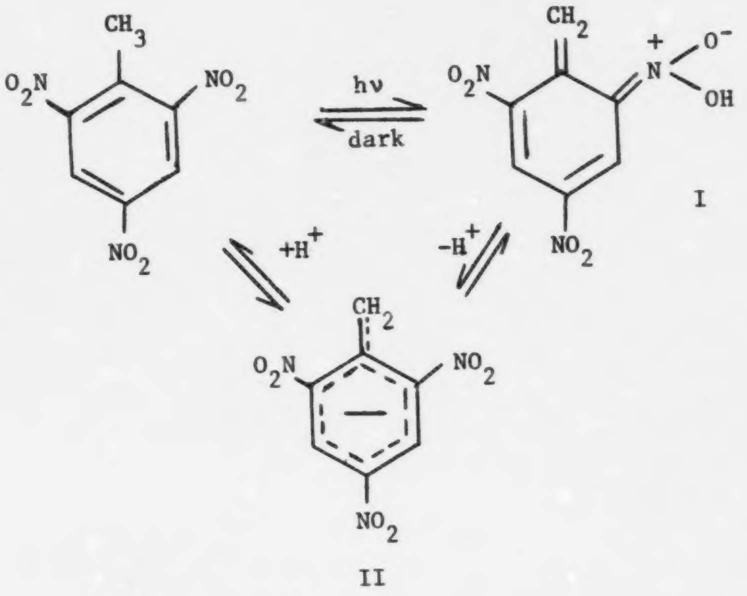


Figure 8. Proposed Mechanism for the Primary Photochemical Step for the Photodecomposition of TNT in Water (Burlinson et al., 1973)

In summary, although very few of the commercially important nitroaromatic compounds have been experimentally tested for sensitivity to ultraviolet light, it is likely that many of the compounds may react photochemically. Although prediction of products is extremely difficult, conversion of the nitro group to nitroso, hydroxylamine, amine, and azoxy moieties seems possible based upon the available information.

II. Environmental Exposure Factors

A. Production, Consumption

1. Quantity Produced and Imported

A considerable amount of quantitative information on the production and sales of nitroaromatic compounds is available from the reports of the U.S. International Trade Commission (formerly the U.S. Tariff Commission) (USITC, 1959-1973). The available information is presented in Table 16. Unfortunately, the production and sales quantities are published by USITC only when there are three or more producers, no one or two of which may be predominant. Table 16 covers only a limited number of nitroaromatic chemicals, but it probably has some information on most of the high volume chemicals, since those chemicals usually have several manufacturers. Production trends are more easily visualized from the USITC (1959-1973) information plotted in Figure 9.

The U.S. International Trade Commission also publishes information on the quantity of benzenoid chemicals that are imported into the United States. The statistics are reported annually, but are based only upon importation reported through major U.S. customs districts. Therefore, the quantities reported are somewhat lower than the actual total material imported. (For 1973, coverage is estimated to be 68% for flavors and perfumes, 78% for drugs, 79% for pigments, 83% for intermediates, and 94% for dyes). Table 17 presents the published statistics on quantities of nitroaromatic compounds that were imported.

From the available published data, as well as from information obtained during discussions with the chemical producers, a list of nitroaromatic

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Table 16. U.S. Production and Sales of Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973)

(1000 lbs)

		1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Nitroaromatic Hydrocarbons																
Nitrobenzene	P*	172,133	162,308	184,558	199,587	219,971	239,216	280,341	326,853	347,700	397,937	484,457	547,680	444,869	33[illegible],169	308,667
	S**	6,897	6,171	6,686	9,095	9,421	9,513	11,506	13,612	12,623	11,450	9,860	---	16,756	12,622	---
p-Nitrotoluene	P	---	---	---	---	---	---	---	---	---	17,750	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
2,4-Dinitrotoluene	P	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	6,517	---	---	---	---	---	---	---
2,4-(and 2,6-) Dinitrotoluene	P	---	---	---	---	---	---	---	---	---	---	256,583	296,503	352,746	433,885	471,237
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Nitroxylenes, mixed	P	545	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Chloronitroaromatic Hydrocarbons																
1-Chloro-2-nitrobenzene	P	---	24,540	17,177	---	18,879	20,088	28,290	36,226	34,226	---	---	---	---	---	---
	S	---	---	---	---	10,715	9,648	10,536	12,315	12,402	14,623	20,391	---	---	---	---
1-Chloro-3-nitrobenzene	P	---	---	---	3,890	6,435	8,063	---	7,908	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
1-Chloro-4-nitrobenzene	P	---	---	---	---	---	86,902	109,757	121,735	101,508	---	---	88,854	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
o- and p-Chloronitrobenzene mixture (1-chloro and 2 and 4-nitro)	P	7,031	10,037	7,494	4,852	3,920	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
1-Chloro-2,4-dinitrobenzene	P	7,851	5,324	6,761	7,217	8,164	8,193	8,107	8,535	6,257	6,626	---	---	---	---	---
	S	---	---	---	1,208	1,580	1,499	1,659	1,508	1,768	2,192	---	---	---	---	---
1,4-Dichloro-2-nitrobenzene	P	---	---	628	276	396	417	705	793	623	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	32	---	---	---	---	---	---

* - P = Production
** - S = Sales

Table 16. U.S. Production and Sales of Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973) (Cont'd)

(1000 lbs)

		1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Chloronitroaromatic Hydrocarbons (Cont'd)																
4-Chloro-2-nitrotoluene	P*	---	---	---	313	696	396	---	---	---	---	---	---	---	---	---
	S**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4-Chloro-3-nitrotoluene	P	---	---	---	71	---	---	---	102	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Nitrophenols and Realted Compounds																
p-Nitrophenol	P	---	---	---	13,093	16,161	18,935	---	---	---	---	---	---	---	---	---
	S	---	---	---	5,618	---	---	---	---	---	---	---	---	---	---	---
p-Nitrophenol and sodium salt	P	---	---	---	---	---	---	19,856	20,025	15,370	33,594	38,837	32,600	---	---	---
	S	---	---	---	---	---	---	11,273	17,920	15,145	14,451	15,741	19,312	16,158	---	---
2-Amino-4-nitrophenol	P	109	76	60	65	132	98	137	---	102	192	104	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
2,4-Dinitrophenol, tech.	P	598	831	945	1,053	1,035	1,037	935	971	775	863	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	149	---	---	---	---
p-(2,4-Dinitroanilino) phenol	P	41	---	---	---	---	33	360	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Methyl parathion	P	5,987	11,794	18,527	16,156	15,999	18,640	29,111	35,862	33,344	38,163	50,572	41,353	37,226	51,076	48,890
	S	7,814	10,262	14,265	12,196	19,174	21,713	27,440	29,973	31,919	45,178	32,818	39,869	46,354	52,438	52,450
Ethyl parathion	P	9,180	7,434	8,423	8,786	---	12,786	16,607	19,444	11,361	---	---	15,259	---	---	---
		7,924	7,518	7,423	5,847	8,618	10,338	14,198	15,536	14,573	19,510	---	15,504	---	---	---
Nitroanisoles and Nitroanisidines																
4-Nitro-o-anisidine	P	133	91	---	144	83	73	103	---	---	---	---	---	---	---	---
	S	18	---	11	26	---	---	---	---	---	---	---	---	---	---	---
5-Nitro-o-anisidine	P	748	325	131	201	284	331	108	250	119	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

* - P = Production

** - S = Sales

Table 16. U.S. Production and Sales of Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973) (Cont'd)

(1000 lbs)

		1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Nitroanilines and Related Compounds																
m-Nitroaniline	P*	201	105	148	159	---	---	---	---	---	---	---	---	---	---	---
	S**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
p-Nitroaniline	P	---	---	---	8,769	9,808	10,890	12,478	10,750	9,001	11,029	---	---	---	---	---
	S	---	---	---	6,922	6,890	7,876	6,883	---	---	---	---	---	---	---	---
2-Chloro-4-nitroaniline	P	412	361	426	297	289	301	448	389	275	348	---	---	---	---	---
	S	---	---	---	---	267	226	---	249	221	355	---	---	---	---	---
4-Chloro-2-nitroaniline	P	448	312	315	172	413	---	461	566	503	---	---	---	---	---	---
	S	279	151	180	219	249	---	---	391	463	491	---	---	---	---	---
2,6-Dichloro-4-nitroaniline	P	19	41	56	81	172	225	259	607	---	---	---	---	---	---	---
	S	---	---	---	---	97	185	---	431	---	---	---	---	---	---	---
2-Bromo-4,6-dinitroaniline	P	---	---	---	---	---	---	---	---	---	112	147	---	258	626	944
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	85	---
2-4-Dinitroaniline	P	---	---	---	---	---	---	---	206	187	207	164	196	---	---	---
	S	---	---	---	---	---	---	---	100	94	111	66	135	88	---	---
4-Nitro-o-toluidine	P	---	12	---	---	11	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
5-Nitro-o-toluidine	P	337	165	176	326	300	358	---	367	156	218	277	99	137	397	353
	S	86	---	---	234	---	---	---	183	192	179	199	95	123	---	312
2-Nitro-p-toluidine	P	1,573	1,291	1,152	1,204	1,195	941	1,257	1,208	864	---	---	---	---	---	---
	S	706	603	602	581	633	643	822	---	---	---	---	---	---	---	---
3'-Nitroacetanilide	P	---	---	---	---	---	---	---	---	---	---	---	15	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4'-Nitroacetanilide	P	---	---	---	---	---	---	---	---	---	---	---	277	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
3',4-Dinitrobenzanilide	P	---	---	---	---	---	---	---	---	---	15	16	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

* - P = Production
** - S = Sales

Table 16. U.S. Production and Sales of Nitroaromatic Compounds (U.S. International Trade Commission, (1959-1973) (Cont'd)

(1000 lbs)

		1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Nitroaromatic Acids and Related Compounds																
m-Nitrobenzoic acid and sodium salt	P*	---	---	---	---	259	255	---	---	---	351	911	---	---	---	---
	S**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
m- and p-Nitrobenzoic acids	P	650	---	---	---	---	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
o-(4-Chloro-3-nitrobenzoyl)-benzoic acid	P	109	157	96	106	134	93	145	220	147	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
m-Nitrobenzenesulfonic acid	P	---	---	2,011	---	---	---	---	---	---	---	---	---	---	---	---
	S	---	---	1,332	---	---	---	---	---	---	---	---	---	---	---	---
m-Nitrobenzenesulfonic acid and sodium salt	P	1,472	2,519	---	1,603	2,092	3,090	2,293	3,711	3,090	3,464	3,081	3,654	---	---	---
	S	1,386	1,388	---	1,608	2,065	2,118	2,397	2,705	2,551	2,289	1,447	2,165	---	---	---
2-Chloro-5-nitrobenzenesulfonic acid and sodium salt	P	270	127	245	---	---	---	---	---	368	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
2-Chloronitrobenzenesulfonic acid	P	---	---	---	---	---	---	89	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4-Chloro-3-nitrobenzenesulfonic acid	P	102	163	180	---	190	---	---	---	---	174	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
2-Amino-5-nitrobenzenesulfonic acid	P	32	59	54	42	56	36	48	72	33	42	49	39	23	---	---
	S	---	---	9	---	---	---	---	---	---	---	---	---	---	---	---
2-(p-Aminoanilino)-5-nitrobenzenesulfonic acid	P	42	---	75	89	---	33	---	---	18	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
5-Amino-2(p-aminoanilino)-benzenesulfonic acid	P	14	22	12	26	24	15	21	7	4	---	11	10	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
3-Nitro-p-toluenesulfonic acid	P	276	90	---	75	86	---	73	87	67	81	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

* - P = Production

** - S = Sales

Table 16. U.S. Production and Sales of Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973) (Cont'd)

(1000 lbs)

		1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
Nitroaromatic Acids and Related Compounds (Cont'd)																
5-Nitro-o-toluenesulfonic acid	P*	3,730	3,399	3,894	4,870	5,403	6,680	8,429	11,261	10,419	6,735	12,911	9,025	7,164	8,017	7,955
	S**	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
7-(and 8-)Nitronaphth[1,2d]-oxadiazole-5-sulfonic acid	P	---	---	497	1,184	913	758	1,084	969	278	676	551	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
3-Nitro-1,5-napthalene-disulfonic acid	P	---	---	---	---	128	207	223	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4-Amino-4'-nitro-2,2'-stilbenedisulfonic acid	P	---	---	---	---	---	---	---	51	145	200	201	264	---	---	245
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4,4'-Dinitrostilbene-2,2'-disulfonic acid	P	2,256	1,967	2,352	2,885	3,423	4,159	6,449	9,376	11,443	11,319	14,682	10,161	10,953	9,230	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
m-Nitrobenzenesulfonyl chloride	P	---	---	---	---	23	---	---	---	---	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4-Chloro-3-nitrobenzene-sulfonyl chloride	P	76	177	129	165	141	235	248	500	553	390	487	426	345	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
4-Chloro-3-nitrobenzene-sulfonamide	P	89	167	139	130	164	258	275	320	420	372	556	431	503	507	743
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Miscellaneous Nitroaromatic Compounds																
4'-(p-Nitrophenyl)-acetophenone	P	---	---	---	---	---	---	---	---	---	---	---	42	---	---	---
	S	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Dinitrobutylphenol, ammonium salt	P	---	---	---	---	---	---	---	85	58	---	---	---	---	---	---
	S	---	---	---	---	---	---	---	70	66	---	---	---	---	---	---

* - P = Production
** - S - Sales

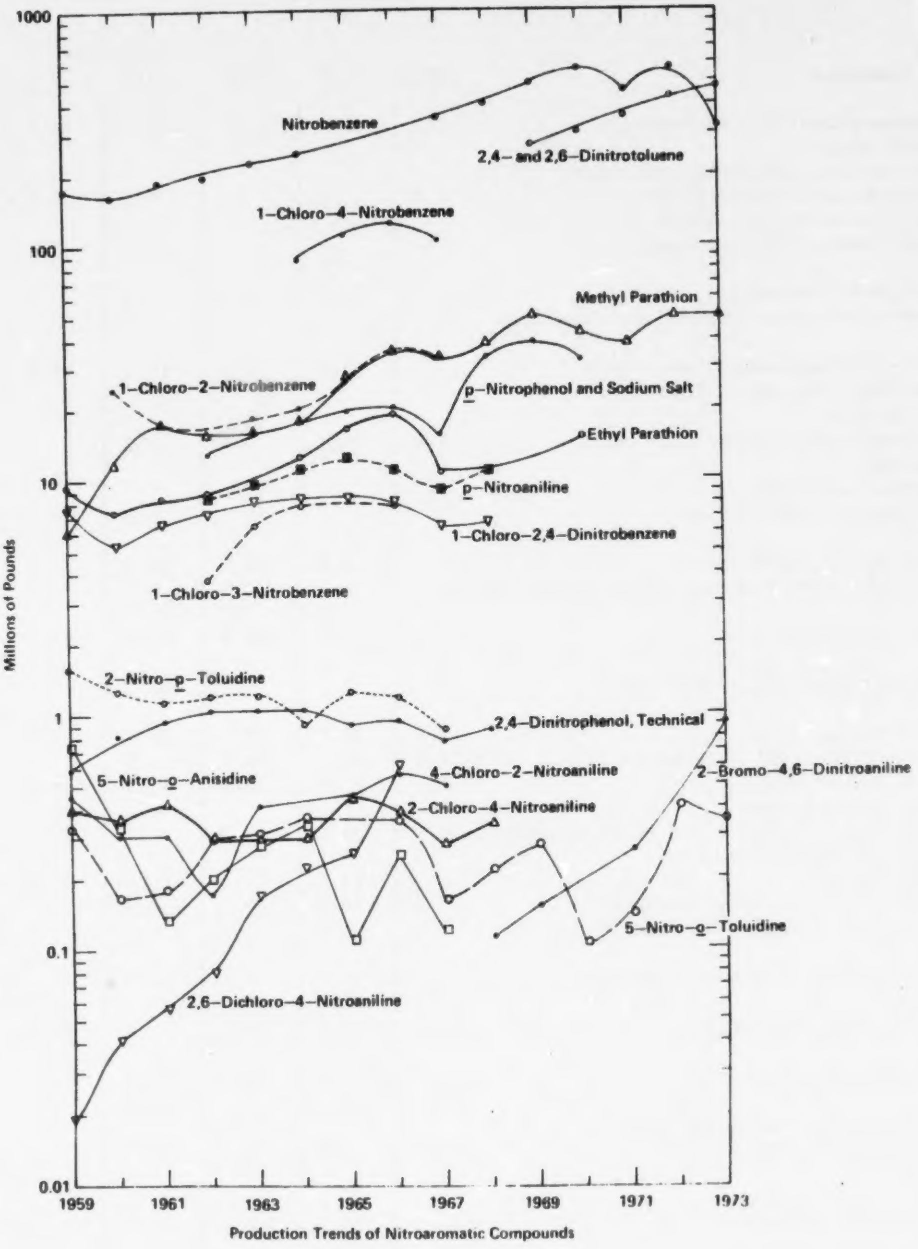

Figure 9. U.S. Production Trends of Nitroaromatic Compounds

Table 17. Imports of Nitroaromatic Chemicals (U.S. International Trade Commission, 1967-1973)

Compound	1967	1968	1969	(1000 lbs) 1970	1971	1972	1973
2-(p-Aminoanilino)-5-nitrobenzene-sulfonic acid	30.1	43.9	28.0	29.3	67.3	15.5	88.6
2-Amino-3-chloro-5-nitrobenzonitrile						4.8	
2-Amino-4-chloro-5-nitrophenol	1.9	3.5		1.5	4.6		
2-Amino-4-chloro-6-nitrophenol						4.4	
2-Amino-6-chloro-4-nitrophenol		3.0		1.3		2.1	2.8
2-Amino-4,6-dinitrophenol	0.7	0.7	0.7	0.8	0.4		
2-Amino-N-ethyl-5-nitrobenzenesulfonanilide		2.2		1.3		2.2	1.2
2-Amino-5-nitrobenzenesulfonic acid		1.8	14.8	11.3	16.8	43.0	51.1
2-Amino-5-nitrobenzenesulfonic acid, ammonium salt							2.1
2-Amino-5-nitrobenzenesulfonic acid, sodium salt	8.6	14.3	4.4	2.2			3.8
2-Amino-5-nitrobenzoic acid			0.1				
2-Amino-5-nitrobenzonitrile				0.6	40.2	129.7	83.9
2-Amino-6-nitrobenzothiazole		0.4	11.5	30.0	24.8	125.8	149.9
2-Amino-5-nitro-N-(phenethyl)benzenesulfonamide			4.3	0.8			
2-Amino-4-nitrophenol		0.5	138.1	48.4	141.5	29.9	118.0
2-Amino-4-nitrophenol, sodium salt				107.3		148.2	90.1
2-Amino-5-nitrophenol	11.6	30.3	12.8	26.2	23.8	49.8	34.7
4-Amino-4'-nitro-2,2'-stibenedisulfonic acid	39.8	21.2	46.7				
2-Bromo-4,6-dinitroaniline		28.9	10.3	47.9	155.1	77.2	92.5
1-Bromo-2-nitrobenzene				0.1	0.7	1.6	
4-t-Butyl-2,6-dimethyl-3,5-dinitroacetophone	26.8	22.8	15.5	23.1	37.5	25.9	42.6
2-sec-Butyl-4,6-dinitrophenol							433.2
6-t-Butyl-3-methyl-2,4-dinitroanisole	66.9	122.5	85.4	55.3	83.6	118.8	84.4
5-t-Butyl-2,4,6-trinitro-m-xylene	127.0	156.2	90.0	163.4	35.9		49.4
2-Chloro-1,4-dibutoxy-5-nitrobenzene		1.1	1.1	1.1	1.5	0.6	2.2
2-Chloro-1,4-diethoxy-5-nitrobenzene		13.8	6.3	4.3	5.7	37.4	71.8
2-Chloro-4,6-dinitroaniline	8.9	23.3	21.4	12.8	67.0	48.7	170.2
1-Chloro-2,4-dinitrobenzene			88.2	132.3		143.3	20.0
4-Chloro-5-nitro-2-aminophenol			5.8				
2-Chloro-4-nitroaniline	15.2	49.6	54.8	162.3	353.6	409.5	541.0
2-Chloro-5-nitroaniline							1.3
4-Chloro-2-nitroaniline	55.2	25.5	144.7	377.4	50.1		
4-Chloro-3-nitroaniline					1.5	11.9	4.4
4-Chloro-3-nitroanisole	0.3			0.1		0.6	0.5
5-Chloro-2-nitroanisole				0.2			0.2
1-Chloro-2-nitrobenzene					0.3	124.1	315.4

Table 17. Imports of Nitroaromatic Chemicals (U.S. International Trade Commission, 1967-1973) (Cont'd)

Compound	1967	1968	1969	1970 (1000 lbs)	1971	1972	1973
2-Chloro-4-nitrobenzoic acid	0.6						
4-Chloro-3-nitrobenzoic acid	22.0	0.1				3.3	30.9
2-Chloro-4-nitrophenol					0.5		
4-Chloro-3-nitrotoluene					1.3		2.2
4-Chloro-α,α,α-trifluoro-3-nitrotoluene					10.3	9.9	10.4
2,6-Dichloro-4-nitroaniline			2.1	16.7	3.3		60.4
1,3-Dichloro-4-nitrobenzene			0.2	0.2			0.9
1,4-Dimethoxy-2-nitrobenzene		1.0					
2,4-Dinitroacetanilide			4.4	2.2	2.2		
2,4-Dinitroaniline	129.4		105.8	273.9	358.8	679.7	596.9
m-Dinitrobenzene					10.1		
3,5-Dinitrobenzoic acid, tech	11.0		29.9	49.9	29.9	89.9	30.1
3,5-Dinitrobenzoyl chloride, tech	0.1		0.05		0.2		0.2
Dinitrobutylphenol						657.7	
4,6-Dinitro-*o*-cresol	42.9	55.0		6.4	19.7	217.9	146.6
2,4-Dinitro-6-methylphenol							2.2
2,4-(and2,6)-Dinitrophenol	2.3						
4,4'-Dinitro-2,2'-stilbenedisulfonic acid	434.5	284.8	441.6	732.8	312.8	628.5	311.9
2,4-Dinitrotoluene	19.9	15.9					
2,4'-Dinitro-4-trifluoromethyldiphenyl ether			39.9	147.2		70.2	
2-(Methylsulfonyl)-4-nitroaniline			7.2	82.6			
m-Nitroaniline	55.6	56.3	112.7	88.2	117.2	97.4	191.6
o-Nitroaniline			75.9	75.4			
p-Nitroaniline	20.2	116.0	32.0	98.1	278.6	24.2	2963.6
2-Nitro-*p*-anisidine	13.0	20.0	15.0	30.1	53.0	27.0	69.8
4-Nitro-*o*-anisidine	11.0		3.3	8.5	6.0		345.3
5-Nitro-*o*-anisidine	9.3	19.2	21.0	34.5	113.2	94.4	127.3
o-Nitroanisole			165.8	114.3		5.0	
m-Nitrobenzaldehyde	2.3	4.4	1.2	6.1	9.9		1.0
m-Nitrobenzenesulfonic acid, sodium salt	252.1	42.9				120.0	237.4
m-Nitrobenzoic acid	219.8	197.9	15.5	29.9	99.9	270.4	108.2
o-Nitrobenzoic acid	1.7	7.2		7.2	7.5	0.7	3.4
p-Nitrobenzoic acid		183.4	29.9				
m-Nitrobenzoyl chloride				30.3		9.4	0.1
p-Nitrobenzoyl chloride	13.2	2.2	20.9	80.2	112.8	238.9	232.9

Table 17. Imports of Nitroaromatic Chemicals (U.S. International Trade Commission, 1967-1973) (Cont'd)

Compound	(1000 lbs) 1967	1968	1969	1970	1971	1972	1973
4-Nitro-m-cresol				124.3			0.04
4-Nitrodiphenylamine							459.6
5-Nitro-1-diazo-2-naphthol-4-sulfonic acid	33.6	49.8	33.1	58.3	47.7	62.1	16.7
3-Nitro-p-phenetidine			3.8		31.6		7.4
4-Nitro-o-phenetidine						2.0	
5-Nitro-o-phenetidine					2.5	4.0	
5-Nitro-p-phenetidine							1.5
m-Nitrophenol			0.2				
o-Nitrophenol		17.7	11.6	5.5	19.1	21.6	19.1
p-Nitrophenol	319.3			187.6	534.3	1610.3	2237.0
2-Nitro-p-phenylenediamine	6.4	0.8	0.6	0.5	0.4	0.6	3.2
4-Nitro-m-phenylenediamine	0.1		0.6		0.1	1.1	0.9
4-Nitro-o-phenylenediamine						0.5	4.2
1-(m-Nitrophenyl)-5-oxo-2-pyrazoline-3-carboxylic acid					2.9	4.6	3.4
p-Nitrophenylphosphate, disodium salt			0.2			0.2	0.06
p-Nitrotoluene	200.9	50.1	18.0				
2-Nitro-m-toluic acid						4.4	
3-Nitro-o-toluic acid				0.1			
3-Nitro-p-toluic acid						1.8	0.6
2-Nitro-p-toluidine							50.9
4-Nitro-o-toluidine	33.1	0.4	1.1	8.2	134.4	334.1	
5-Nitro-o-toluidine	15.2	9.9	31.5	13.2	4.6	7.4	2.4
p-Nitro-o-xylene						407.1	
Pentachloronitrobenzene	30.0	20.0	132.4				
Trinitrotoluene			100.0				

compounds that are produced annually in over 500,000 lbs was developed. The compounds are listed in descending order of production volume in Table 18. In some cases, the information may not be exact, but the relative magnitudes of production volumes are believed to be accurate. From Table 18 it is apparent that only about a half dozen nitroaromatic compounds are produced in truly large scale. (Austin (1974) suggests 50 million lbs/year as a reasonable breaking point between large scale and modest production.) Furthermore, some of the large production compounds are related by synthesis to other large volume nitroaromatic chemicals. For example, 50% of p-chloronitrobenzene is consumed in the production of p-nitrophenol, and p-nitrophenol is in turn consumed (87% of the total) in the production of methyl and ethyl parathion.

2. Producers and Production Sites

The Chemical Index contains a complete list of commercial nitroaromatic compounds with producers and production sites. Table 19 contains information on producers and plant capacities and locations only for the major nitroaromatic compounds listed in Table 18.

3. Production Methods and Processes

a. General

Although nitroaromatic compounds may be produced by a number of synthetic steps, the one common reaction that they all undergo is direct nitration with a combination of nitric and sulfuric acids (mixed acid). This step may occur in a variety of positions in the synthesis sequence (see Section I-B-1, p. 21 for synthesis strategies). Both batch and continuous operations are used for the nitration step and the procedure used is dependent upon the quantity produced. Production methods for some

Table 18. Production Volumes of Major Commercial Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973, 1967-1973; Chemical Marketing Reporter, 1969, 1972, 1973 a, 1974 a, b; Industry Sources)

Compound	Approximate Volume millions of pounds	Most Recent Statistics	Reference
Nitrobenzene	655,000 (demand, 1974)		CMR (1974b)
2,4-(and 2,6)Dinitrotoluene	471,237 (production, 1973)	Same	USITC (1959-73)
2,4,6-Trinitrotoluene	432,000 (production, late 1973)		Rosenblatt *et al*. (1973)
1-Chloro-4-nitrobenzene	110,000 (production)	107,000 (1974)	Industry sources CMR (1974a)
p-Nitrophenol and sodium salt	60-100,000	35,000 demand (1972)	Industry sources CMR (1972)
1-Chloro-2-nitrobenzene	60,000	34,226 (production, 1967)	Industry sources USITC (1959-73)
O-O-Dimethyl-*o*,*p*-nitrophenylphosphorothioate (methyl parathion)	48,890 (production, 1973)		USITC (1959-73)
α, α, α-Trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine (Trifluralin)	25,000 (production, 1972)		Lawless *et al*. (1972)
p-Nitrotoluene	20-25,000	17,750 (production, 1968)	Industry sources USITC (1959-73)
O-O-Diethyl-*o*,*p*-nitrophenylphosphorothioate (Parathion)	15,259 (production, 1970)		USITC (1959-73)
o-Nitrophenol	10-15,000		Industry sources
p-Nitroaniline	~14,000	11,029 (production, 1968) 10,500 (demand, 1973)	Industry sources USITC (1959-73) CMR (1969)

Table 18. Production Volumes of Major Commercial Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973, 1967-1973; Chemical Marketing Reporter, 1969, 1972, 1973 a, 1974 a, b; Industry Sources) (Cont'd)

Compound	Approximate Volume millions of pounds	Most Recent Statistics	Reference
1,3-Dinitrobenzene	∼12,000		Starr (1972)
o-Nitrotoluene	10-12,000		Industry sources
4,4'-Dinitrostilbene-2,2'-disulfonic acid	9,230 (production, 1972)		USITC (1959-73)
5-Nitro-*o*-toluenesulfonic acid	7,955 (production, 1973)		USITC (1959-73)
1-Chloro-3-nitrobenzene	7,908 (production, 1966)		USITC (1959-73)
1-Chloro-2,4-dinitrobenzene	6,626 (production, 1968)		USITC (1959-73)
1-Nitronaphthalene	6,290 (production, 1972)		Industry sources
o-Nitroaniline	6,000		Industry sources
m-Nitrobenzenesulfonic acid and sodium salt	3,654 (production, 1970)		USITC (1959-73)
1,2-Dichloro-4-nitrobenzene	3,000-3,600		Industry sources
2-*sec*-Butyl-4,6-dinitrophenol	3,000		Lawless *et al*. (1972)
2-Chloro-4-nitroaniline	2,500-3,000	541 (import, 1973)	Industry sources USITC (1967-73)
o-Nitroanisole	2,500-3,000		Industry sources
p-Nitroanisole	750-1,500		Industry sources
2,4-Dinitrophenol	1,000	863 (production, 1968)	Industry sources USITC (1959-73)
2-Bromo-4,6-dinitroaniline	944 (production, 1973)		USITC (1959-73)

Table 18. Production Volumes of Major Commercial Nitroaromatic Compounds (U.S. International Trade Commission, 1959-1973, 1967-1973; Chemical Marketing Reporter, 1969, 1972, 1973 a, 1974 a, b; Industry Sources) (Cont'd)

Compound	Approximate Volume millions of pounds	Most Recent Statistics	Reference
m-Nitrobenzoic acid and sodium salt	911 (production, 1969)		USITC (1959-73)
2-Nitro-*p*-toluidine	864 (production, 1967)		USITC (1959-73)
1,4-Dichloro-2-nitrobenzene	700-800	623 (production, 1967)	USITC (1959-73)
4-Chloro-3-nitrobenzenesulfonamide	743 (production, 1973)		USITC (1959-73)
4-Chloro-2-nitrotoluene	696 (production, 1963)	396 (production, 1964)	USITC (1959-73)
2,4-Dinitroaniline	≥679 (import, 1972)		USITC (1967-73)
2,6-Dichloro-4-nitroaniline	607 (production, 1966)		USITC (1959-73)
2-Chloro-5-nitrobenzenesulfonic acid and sodium salt	500-600	368 (production, 1967)	Industry sources USITC (1959-73)
7-(and 8)Nitronaph[1,2]oxadiazole-5-sulfonic acid	551 (production, 1969)		USITC (1959-73)
3,5-Dinitrobenzoic acid	∿500		Industry sources

Table 19. Major Nitroaromatic Compound Producers, Capacities, and Plant Locations

Compound	Manufacturer	Plant Location	Annual Capacity (10^6 lbs)	Reference
Nitrobenzene	Allied Chemical	Moundsville, WV	55	
	American Cyanamid	Bound Brook, NJ	85	Chemical Marketing Reporter (1974b)
		Willow Island, WV	60	
	DuPont	Gibbstown, NJ	200	SRI (1975)
		Beaumont, TX	310	
	First Chemical	Pascagoula, MS	135	
	Mobay	New Martinsville, WV	135	
	Monsanto	Sauget, IL	10	
	Rubicon	Geismar, LA	75	
2,4-(and 2,6)Dinitrotoluene	DuPont	Deepwater, NJ		
	Mobay	Cedar Bayou, TX		
		New Martinsville, WV		
2,4,6-Trinitrotoluene (TNT)	government-owned contractor-operated facilities	Radford, VA	108	Nay, 1972
		Newport, IN	180	Forsten (1973)
		Chattanooga, TN	360	Rosenblatt et al., (1973)
		Joliet, IL	432	
1-Chloro-4-nitrobenzene	DuPont	Deepwater, NJ	45 (90 by 1977)	Chemical Marketing Reporter (1974a)
	Monsanto	Sauget, IL	95	Anon. (1975a) Anon. (1975b)
p-Nitrophenol and sodium salt	DuPont	Deepwater, NJ	15	Chemical Marketing Reporter (1972)
	Martin Marietta	Sodyeco, NC	1	
	Monsanto	Anniston, AL	24	SRI (1975)
		Sauget, IL	12	
	Northern Chem	Franklin, NJ	--	
	Searle	Norwood, OH	--	
1-Chloro-2-nitrobenzene	American Color & Chem.	Lock Haven, PA		SRI (1975)
	DuPont	Deepwater, NJ	(see 1-chloro-4-nitrobenzene)	
	Monsanto	Sauget, IL		

Table 19. Major Nitroaromatic Compound Producers, Capacities, and Plant Locations (Cont'd)

Compound	Manufacturer	Plant Location	Annual Capacity (10^6 lbs)	Reference
Methyl[a] and ethyl[b] parathion	Hercules	Plaquemine, LA	15[a] (not operating)	SRI (1975)
	Kerr-McGee	Hamilton, MS	17[a]	
	Monsanto	Anniston, AL	50[a,b]	
	Stauffer	Mt. Pleasant, TN	30[a,b]	
	Vicksburg Chem.	Vicksburg, MS	--	
Trifluralin	Eli Lilly	Lafeyette, IN		
p-Nitrotoluene	DuPont	Deepwater, NJ		SRI (1975)
	First Mississippi	Pascagoula, MS		
o-Nitrophenol	Martin-Marietta	Sodyeco, NC		SRI (1975)
	Monsanto	Sauget, IL		
p-Nitroaniline	American Color & Chem.	Lock Haven, PA	2.0	SRI (1975)
	Monsanto	Sauget, IL	10.0	
	Universal Oil Prod.	McCook, IL	3.0	
m-Dinitrobenzene	DuPont	Deepwater, NJ		USITC (1959-73)
o-Nitrotoluene	DuPont	Deepwater, NJ		SRI (1975)
	First Mississippi	Pascagoula, MS		
4,4'-Dinitrostilbene-2,2'-disulfonic acid	American Cyanamid	Bound Brook, NJ		SRI (1975)
	Ciba-Geigy	McIntosh, AL		
	GAF	Rensselaer, NY		
	Toms River	Toms River, NJ		
5-Nitro-o-toluenesulfonic acid	American Cyanamid	Bound Brook, NJ		SRI (1975)
	DuPont	Deepwater, NJ		
	GAF	Rensselaer, NY		
	Toms River	Toms River, NJ		
1-Chloro-3-nitrobenzene	GAF	Linden, NJ		SRI (1975)
1-Chloro-2,4-dinitrobenzene	Martin-Marietta	Sodyeco, NJ		SRI (1975)

Table 19. Major Nitroaromatic Compound Producers, Capacities, and Plant Locations (Cont'd)

Compound	Manufacturer	Plant Location	Annual Capacity (10^6 lbs)	Reference
1-Nitronaphthalene	DuPont	Deepwater, NJ		Industry sources
o-Nitroaniline	Monsanto	Sauget, IL		SRI (1975)
m-Nitrobenzenesulfonic acid and salts	American Cyanamid GAF USM	Bound Brook (K$^+$ only) Linden, NJ (Na$^+$ only) Greenville, SC (Na$^+$ only)		SRI (1975)
1,2-Dichloro-4-nitrobenzene	Blue Spruce DuPont	Edison, NJ Deepwater, NJ		SRI (1975) Industry Sources
2-*sec*-Butyl-4,6-dinitrophenol	Blue Spruce Dow Chemical Vicksburg Chem.	Edison, NJ Midland, MI Vicksburg, MS		SRI (1975)
2-Chloro-4-nitroaniline	Chemetron DuPont	Huntington, WV Deepwater, NJ		SRI (1975)
o-Nitroanisole	DuPont Monsanto	Deepwater, NU St. Louis, MO		SRI (1975)
p-Nitroanisole	DuPont	Deepwater, NJ		SRI (1975)
2-Bromo-4,6-dinitroaniline	American Color & Chem. Martin-Marietta Toms River	Lock Haven, PA Sodyeco, NC Toms River, NJ		SRI (1975)
m-Nitrobenzoic acid and sodium salt	Bofors Salisbury Sterling Drug	Linden, NJ Charles City, IA Cincinnati, OH		SRI (1975)
2-Nitro-*p*-toluidine	Sherwin-Williams	Chicago, IL		SRI (1975)
2,4-Dinitrophenol	Martin-Marietta	Sodyeco, NC		SRI (1975)

Table 19. Major Nitroaromatic Compound Producers, Capacities, and Plant Locations (Cont'd)

Compound	Manufacturer	Plant Location	Annual Capacity (10^6 lbs)	Reference
1,4-Dichloro-2-nitrobenzene	DuPont	Deepwater, NJ		SRI (1975)
	Mobay	Bayonne, NJ		
4-Chloro-3-nitrobenzenesufonamide	GAF	Rensselaer, NY		SRI (1975)
	Inmont	Hawthorne, NJ		Industry sources
	Salisbury	Charles City, IA		
	Toms River	Toms River, NJ		
4-Chloro-2-nitrotoluene	American Color & Chem.	Lock Haven, PA		SRI (1975)
	Synalloy Corp.	Spartanburg, SC		
2,4-Dinitroaniline	American Color & Chem.	Lock Haven, PA		SRI (1975)
	Martin-Marietta	Sodyeco, NC		
2,6-Dichloro-4-nitroaniline	GAF	Rensselaer, NY		SRI (1975)
	Kewanee Oil	Louisville, KY		
	Upjohn	North Haven, CT		
2-Chloro-5-nitrobenzenesulfonic acid and sodium salt	DuPont	Deepwater, NJ		SRI (1975)
	Toms River	Toms River, NJ		Industry Sources
7-(and 8)Nitronaph[1,2]oxodiazole-5-sulfonic acid	GAF	Rensselaer, NY		SRI (1975)
	Mobay	Bayonne, NJ		
		Charleston, SC		
	Toms River	Toms River, NJ		
3,5-Dinitrobenzoic acid	Ashland Oil	Great Meadows, NJ		SRI (1975)
	Bofors	Linden, NJ		
	Salisbury	Charles City, IA		

of the large production nitroaromatic compounds are reviewed in detail in the following sections, while production methods for the less important commercial chemicals are briefly outlined in Section I-B-1 (see Figure 6, p. 26).

b. Nitrobenzene (Matsuguma, 1967a; Processes Research, Inc., 1972)

Nitration of benzene can be carried out in either a batch or continuous process. The reaction vessels, constructed of cast iron or steel, are jacketed and generally have external cooling coils for maintaining temperature control of the strongly exothermic reactions. Emergency "drown" tanks containing water are also provided, so that a reaction that has gone out of control can be quenched.

The batch equipment is normally sized for 1000-1500 lb quantities of benzene and operates on a 2-4 hour time cycle. A typical run begins by charging the nitrator with benzene and a heel of spent acid, followed by slow addition of the mixed acid (53-60% H_2SO_4, 32-39% HNO_3, and 8% H_2O) under the surface of the benzene. The reaction temperature is maintained at 50-55°C by adjusting the rate of feed, the rate of heat exchange, and the amount of agitation. Near the end of the reaction the temperature is usually raised to 90°C to promote completion. The nitrobenzene is removed from the spent acid by gravity separation. (About 0.5% of the yield of nitrobenzene is lost due to incomplete separation; Matsuguma, 1967a.) The spent acid, which is drawn off from the bottom of the separator, is either recovered or used to start subsequent runs. The crude nitrobenzene can be used directly in the manufacture of aniline (∿ 97% of the nitrobenzene produced is used directly for aniline synthesis). However, if pure nitrobenzene is required, the product is washed with water and dilute sodium carbonate and then distilled.

In newer plants, which usually use a continuous process, the sequence of operation is essentially the same. The main differences are that smaller reaction vessels, lower nitric acid concentrations, and higher reaction rates are used. For comparable production capacity, a 30-gal stainless steel continuous nitrator can be used instead of the 1500-gal batch nitrator. Because a high speed (600 rpm) agitator is used, a reaction time of only 15-20 minutes is required. Typical yields from the continuous reactor are 96-98% of theoretical, compared to 95-98% for batch process nitration. A flow diagram for a representative nitrobenzene plant is presented in Figure 10.

Two other methods which could be used for the production of nitrobenzene have been explored: 1) continuous vapor phase nitration (this eliminates the use of sulfuric acid) and 2) tubular reactors for reaction of aromatic hydrocarbons with mixed acid (yields up to 99.3% are possible). Whether these processes have reached commercial scale yet is unknown.

c. Dinitrotoluene (Processes Research, Inc., 1972)

As with nitrobenzene, dinitrotoluene can be produced by batch or continuous process. The starting material for dinitrotoluene is mononitrotoluene, either 2-nitrotoluene or 4-nitrotoluene, although toluene itself is sometimes used. If 2-nitrotoluene is used, the product will contain the 2,6-dinitrotoluene isomer. The continuous process usually consists of several reactors joined in series. The raw materials are added only to the first reactor with the successive kettles providing additional reaction time. The mixed acid that is used in this process has an approximate composition of 72% H_2SO_4, 17% HNO_3, and 11% H_2O (made from 50-60% HNO_3 and 93% H_2SO_4) and

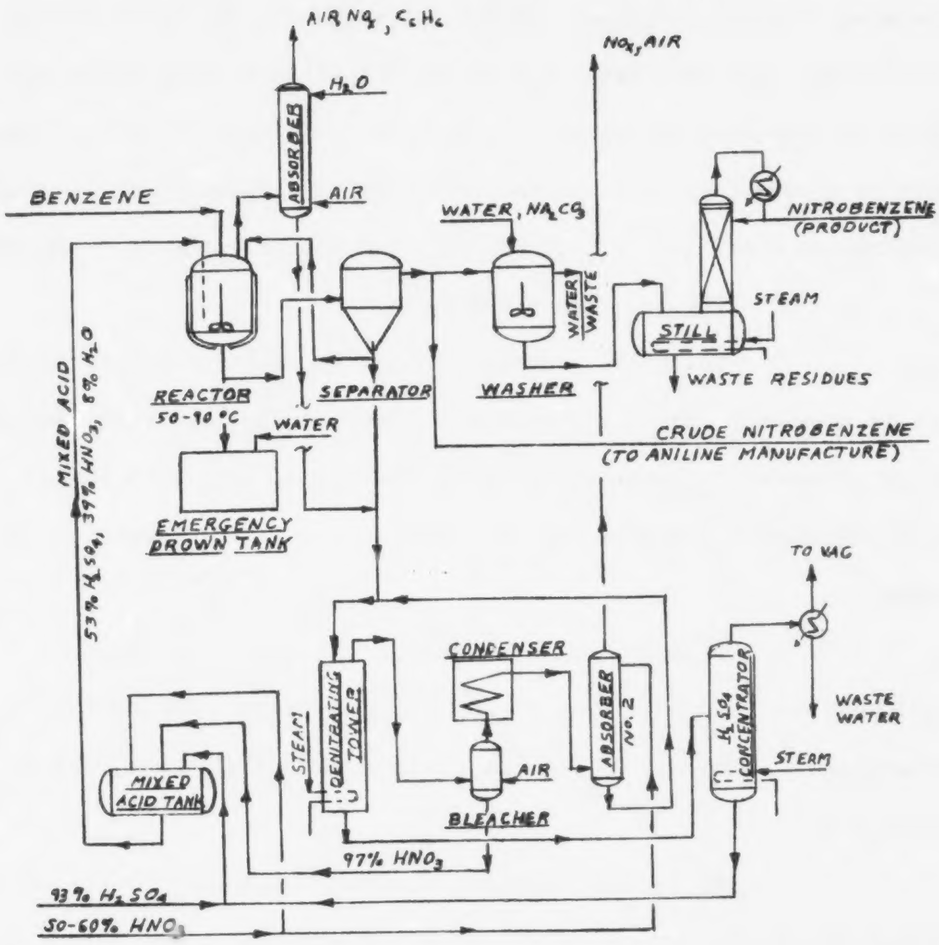

Figure 10. Nitrobenzene Process (Processes Research, Inc., 1972)

the exothermic reaction is maintained at 75-85°C. The overall yield for the reaction is approximately 96%.

The product is removed from the spent acid in a decanter and the spent acid is recycled. In the Meissner process, three washing and neutralization steps are used. A flow sheet for the washing steps and the nitrators is presented in Figure 11. Most of the product that is formed goes directly to a reduction step for the formation of diaminotoluene, but some material may be distilled if high purity 2,4-dinitrotoluene is required.

d. Chloronitrobenzene Process

Chloronitrobenzenes are produced by nitration of chlorobenzene in equipment very similar to that used for the previously-described nitration processes. The reaction is easily carried out, even though the chlorine substituent slightly deactivates the ring to electrophilic substitution reactions.

A typical plant operates with a batch process, using 2640-gal reactors into which are fed 5500 lbs of spent acid and 10,000 lbs of chlorobenzene, followed by 15,600 lbs of mixed acid (53% H_2SO_4, 35% HNO_3, and 12% H_3O).

The crude chloronitrobenzene (process yield is not available) obtained from the nitration process contains about 34% o-chloronitrobenzene, 65% p-chloronitrobenzene, and 1% m-chloronitrobenzene (Matsuguma, 1967a). The isomers are separated by a combination of crystallization and distillation. The crude material is first cooled to 16°C, where 50% of the para-isomer crystallizes out as pure product. The mother liquor is then

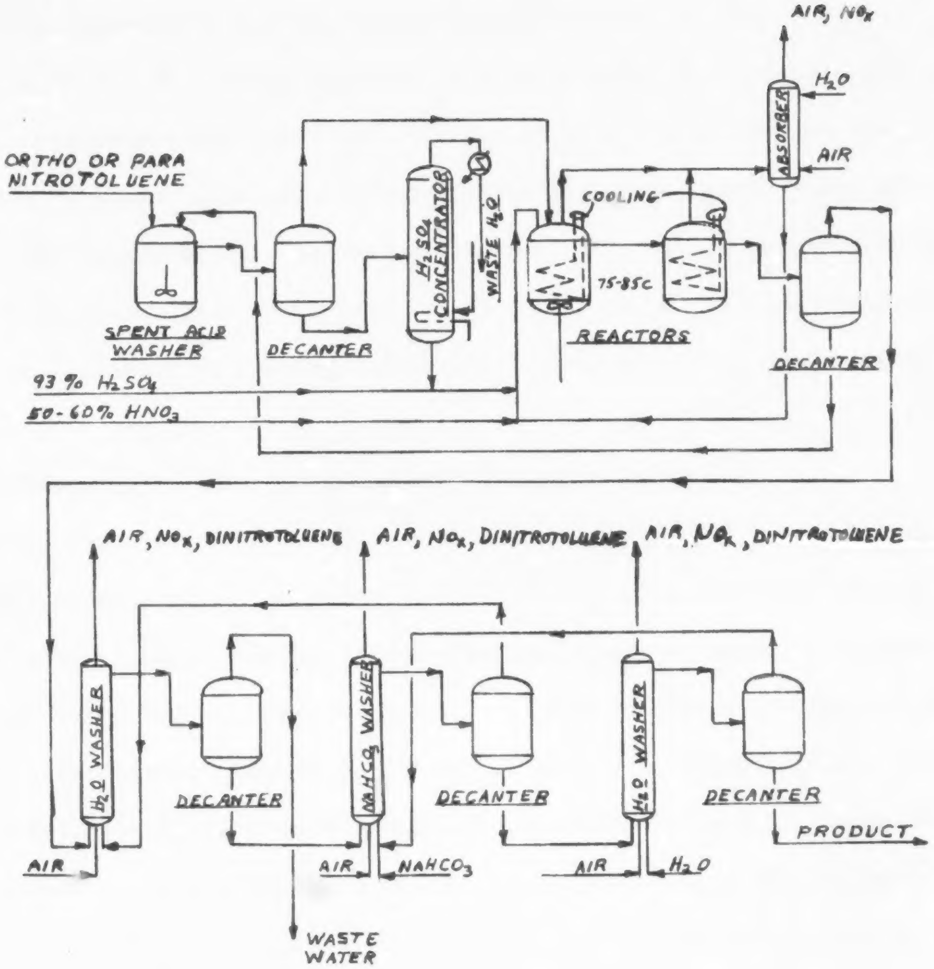

Figure 11. 2,4-Dinitrotoluene Process (Processes Research, Inc., 1972)

fractionally distilled at reduced pressure. The flow and processing of the various cuts are illustrated in Figure 12.

e. Trinitrotoluene Processes

Trinitrotoluene can be prepared by a countercurrent continuous flow process using toluene as a starting material. The usual practice is to nitrate toluene stepwise to mono-, di-, and then trinitrotoluene. Between the three steps, the nitrated toluenes and the mixed acids flow in opposite directions, so that the third stage receives the strongest acid, which becomes weaker in the second stage and weaker still in the first stage. A flow diagram for the countercurrent continuous process is presented in Figure 13.

There are a few differences between the TNT process and the previously described nitration processes. The final TNT product has to have a high degree of isomer purity. (Unsymmetrical isomers cause random explosivity.) In order to remove the undesirable unsymmetrical isomers (non-2,4,6-isomers), a sodium sulfite (sellite) washing is used after the base wash. Sulfite reacts with the unsymmetrical isomers, solubilizing them in the wash water. Another difference is that the amount of 93% sulfuric acid produced in the acid concentrator exceeds what can be used in the process, so this becomes a by-product.

Nay (1972) (see also Rosenblatt *et al.*, 1973) reported that a more automated continuous countercurrent process is presently being used to produce TNT at the Radford Army Ammunition Plant. A major difference is that no "red water" (sellite water) is released as waste. It is recycled as much as possible and then routed for incineration of combustibles

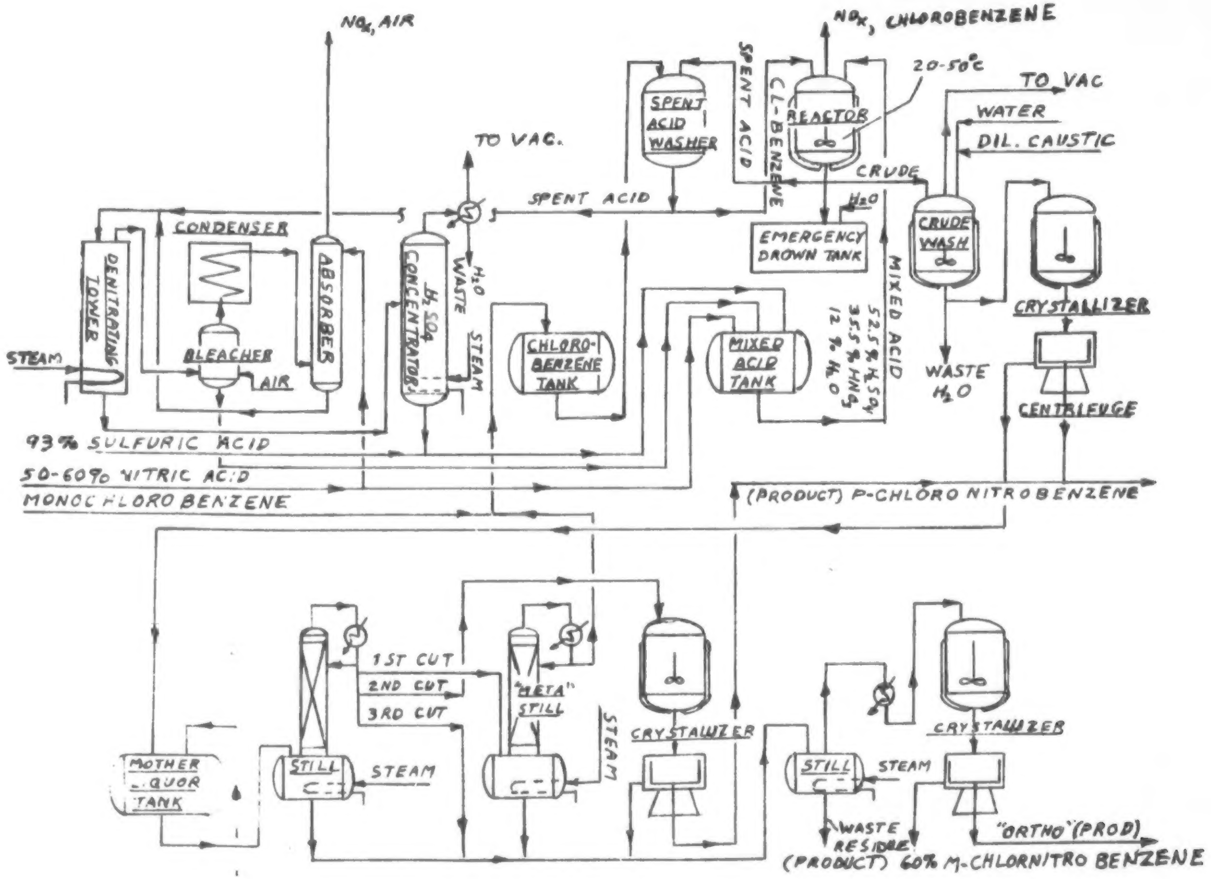

Figure 12. Monochloronitrobenzene Process (Processes Research, Inc., 1972)

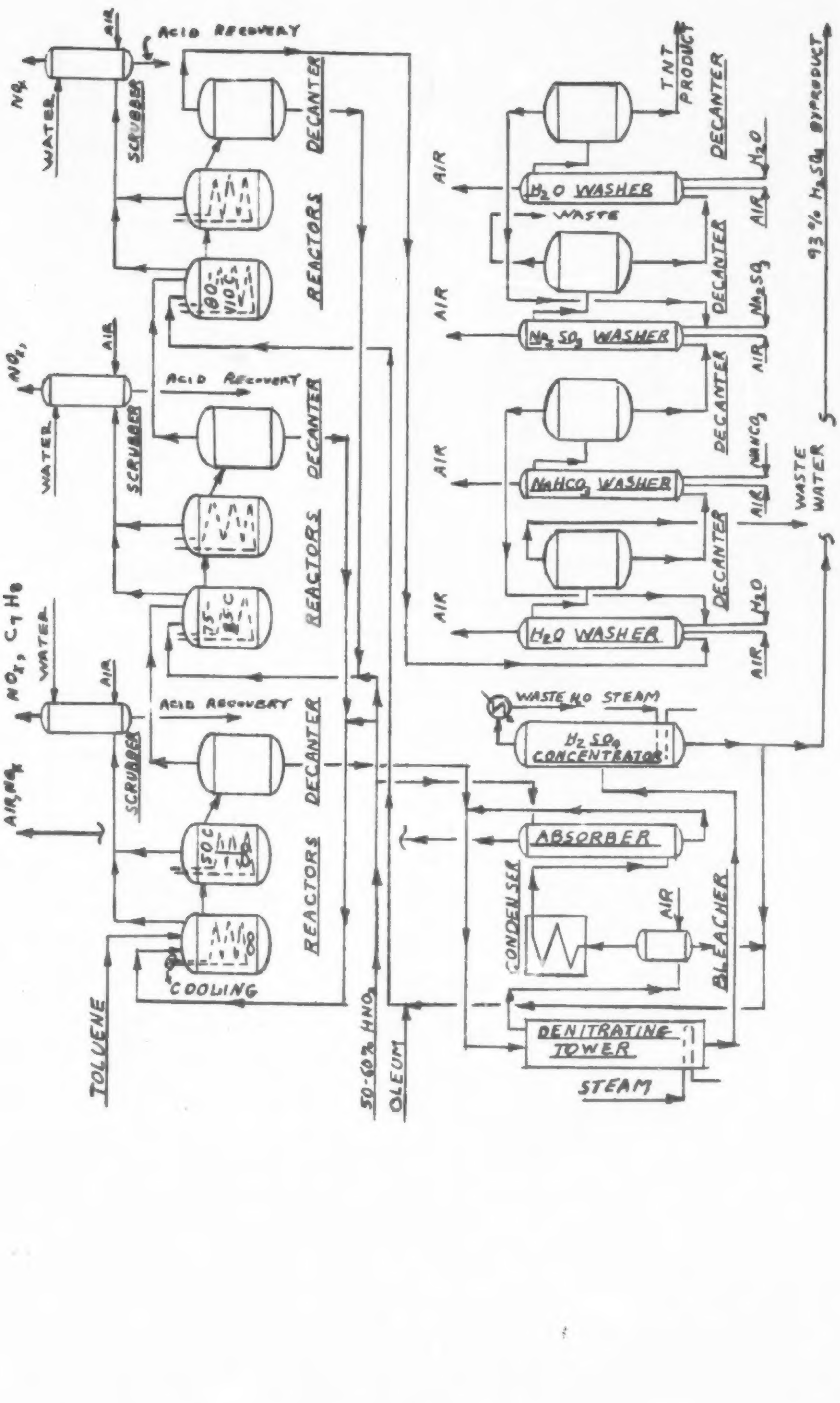

Figure 13. Continuous Countercurrent Trinitrotoluene Process (Processes Research, Inc., 1972)

and oxidation of sodium sulfite to marketable sodium sulfate. With the TNT process, 100 lbs of finished TNT requires about 47 lbs of toluene, 210 lbs of oleum (H_2SO_4), 125 lbs of nitric acid, 6 lbs of sodium sulfite, 1 lb of soda ash, and 650 lbs of water.

Recently, a low temperature TNT process has been reported by Haas *et al*. (1975). The basic feature consists of low-temperature (-8°C) dinitration, followed by higher temperature (90°C) trinitration.

f. Nitrophenol Processes (Matsuguma, 1967b)

Because of the presence of a hydroxyl group on the benzene ring, phenols can be nitrated readily; however, since they are also readily oxidized under nitration conditions, direct nitration is not used commercially to produce nitrophenol. Instead, the commercial process uses hydrolysis of chloronitrobenzenes with aqueous sodium hydroxide at elevated temperatures. For example, when *p*-chloronitrobenzene is heated for four hours at 160°C with 15% aqueous sodium hydroxide, good yields of *p*-nitrophenol are obtained. Generally, only *o*- or *p*-chloronitrobenzene is used to make nitrophenols by hydrolysis because the chlorine substituent has been activated by the nitro group. (This is not the case with *m*-chloronitrobenzene.)

4. Market Prices

Reported prices of nitroaromatic compounds vary considerably and appear to be generally related to the quantity produced. Table 20 compares the price and production volume of a number of nitroaromatic compounds. The compounds in the range of ten cents per pound are those produced in very high quantities. The lower-priced chemicals appear to be simpler chemical entities, suggesting less complex (fewer synthesis steps), and, therefore,

Table 20. Comparative Prices and Production Volumes of Some Nitroaromatic Chemicals (U.S. International Trade Commission, 1959-1973)

Compound	Total Range of Prices Reported ($/lb)	Time Range	Production Range Reported (1000 lbs)
Nitrobenzene	0.11-0.06	1959-72	172,123-551,169
1-Chloro-2-nitrobenzene	0.06-0.09	1963-69	17,177-34,226
p-Nitrophenol	0.42	1962	13,092-18,935
p-Nitroaniline	0.42-0.43	1962, 1965	8,769-12,478
m-Nitrobenzenesulfonic acid and sodium salt	0.34-0.43	1959-70	1,472-3,711
1,4-Dichloro-2-nitrobenzene	0.56	1960	276-793
2,4-Dinitroaniline	0.72-0.79	1967-71	164-207
4-Chloro-2-nitroaniline	0.78-0.84	1959-68	172-566
2-Chloro-4-nitroaniline	0.88-0.93	1963, 1968	275-448
2-Nitro-*p*-toluidine	1.03-1.25	1959-65	864-1,573
2-Amino-5-nitrobenzenesulfonic acid	1.22	1961	23-72
5-Nitro-*o*-toluidine	1.32-1.60	1959-65	99-397
Dinitrobutylphenol, ammonium salt	1.57-1.80	1966-67	58-85
2,6-Dichloro-4-nitroaniline	1.13-1.74	1963-66	19-607
4-Nitro-*o*-anisidine	2.00-2.38	1959-62	73-144

cheaper production processes. Table 21 contains recent market prices for nitroaromatic chemicals.

5. Market Trends

Nitroaromatic compounds are used mostly as chemical intermediates for dyes, pigments, pharmaceuticals, rubber chemicals, photographic chemicals, and agricultural chemicals. These markets appear to be fairly stable, and, as a result, nitroaromatic chemical production and consumption has, in general, remained constant or increased (see Figure 9, p. 53 for production trends).

During 1963-1973, nitrobenzene experienced an average growth rate of 10.6% per year, and a 7% increase per year through 1978 is projected (Chemical Marketing Reporter, 1974 b). Most of this growth is attributed to the growth of aniline, the major (97%) application; the market for aniline continues to grow. Major applications for aniline include isocyanates (40%), rubber chemicals (35%), dye stuffs and intermediates (6%), hydroquinone (6%), drugs (4%), and miscellaneous (9%) (Anon., 1974). The total market for aniline, which was 535 million lbs in 1974, is projected to grow at 9.5% per year through 1977 (Chemical Marketing Reporter, 1973 b).

Dinitrotoluene should also look forward to a growth market because of its use in the production of toluene-2,4-diisocyanate. Toluene diisocyanate accounted for 67% of the total isocyanate production in 1970 (Dean, 1971). From 1965 to 1970, the United States isocyanate production grew by an average of 21% per year and a 12-15% annual growth rate for 1970-1975 was projected. Isocyanates are used almost exclusively to produce polyurethane polymers.

Table 21. Recent Market Prices of Nitroaromatic Chemicals (Chemical Marketing Reporter, 1974 c, 1975 a, b)

Chemical	Price ($/lb)					
	November 4, 1975		April 21, 1975		October 20, 1975	
	Low	High	Low	High	Low	High
2-Chloro-4-nitroaniline						
paste, delivered, East 100% basis	0.95		0.95		0.95	
powder, delivered, East 100% basis	1.05		1.05		1.05	
4-Chloro-2-nitroaniline						
powder, delivered, East	0.86	0.95	0.86	0.95	0.86	0.95
4-Chloro-2-nitrophenol, technical						
paste, drums, freight allowed	0.5		0.75		0.75	
4-Chloro-2-nitrotoluene, technical						
solid, drums, freight allowed	0.99		0.99		0.99	
6-Chloro-2-nitrotoluene, technical						
solid, drums, freight allowed	0.40		0.40		0.40	
2,6-Dichloro-4-nitroaniline						
drums, 10,000 lbs or more, works	1.38		1.38		1.38	
2,4-Dinitroaniline						
drums, delivered	1.20		1.20		1.20	
Dinitroaniline, orange toner						
chemically pure, bags, delivered, East	2.85	3.10	2.85	3.10	2.85	3.00
m-Dinitrobenzene, 89°, technical						
drums, truckload	0.36		0.36		0.36	
2,4-Dinitrochlorobenzene						
47°C, f.o.b., Charlotte, NC	0.53		0.53		0.53	
2,4-Dinitrophenol, drums	0.90		1.04		1.04	
2,4-Dinitrotoluene, drums, carload						
truckload, works	0.24		0.24		0.24	
tanks, works	0.225		0.225		0.225	
Methyl parathion, technical, 80%						
drums, freight allowed, East	0.48	0.50	0.85		0.92	1.00
Musk, synthetic ambrette						
drums, 100 lb lots	3.20		3.20		5.90	6.00
Musk, synthetic, xylol, drums						
100 lb lots	1.08		1.08		2.50	2.60
m-Nitroaniline						
crystalline, drums, freight allowed	1.40		1.40		1.40	
paste, drums, freight allowed, 100%	1.33		1.33		1.33	

Table 21. Recent Market Prices of Nitroaromatic Chemicals (Chemical Marketing Reporter, 1974 c, 1975 a, b) (Cont'd)

Chemical	Price ($/lb)					
	November 4, 1975		April 21, 1975		October 20, 1975	
	Low	High	Low	High	Low	High
o-Nitroaniline						
flake, drums, truck load, works	0.40		--		0.77	
molten, tanks, freight, works	--		0.74		0.74	
orange toner, bags, freight allowed	1.90		--		1.90	
p-Nitroaniline, drums, carlot truckload; 30,000 lbs min., works	0.52		0.84		0.84	
o-Nitroanisole, technical, tanks, works	--		0.75		0.75	
p-Nitroanisole, technical, solid, drums, freight allowed	0.72		0.72		0.72	
Nitrobenzene, double-distilled, tanks, works	0.19	0.22	0.19	0.22	0.19	0.22
p-Nitrobenzoic acid, drums, carlot, truckload, works	0.50		0.50		0.50	
o-Nitrochlorobenzene						
drums, carlot, freight allowed	0.39		0.49		0.49	
tanks, same basis	0.34		0.42		0.42	
p-Nitrochlorobenzene						
drums, carlot, truckload, works	0.39		0.43		0.43	
tanks, same basis	0.34		0.38		0.38	
2-Nitro-p-cresol, technical, drums, truckload, freight allowed	0.71		0.71		0.71	
o-Nitrophenol, drums, f.o.b., works	0.45		0.45		0.45	
tanks, same basis	0.43		0.43		0.43	
drums, less truckload, freight allowed	0.47		0.47		0.47	
m-Nitrotoluene, technical, drums, freight allowed	0.60		0.60		0.60	
o-Nitrotoluene, drums, carlot, freight allowed	0.16		0.14		0.16	
tanks, freight allowed	0.14		0.14		0.14	
p-Nitrotoluene, technical, drums, carlot, works	0.27		0.27		0.27	
tanks, works	0.20		0.20		0.20	
2-Nitro-p-toluidine, drums, f.o.b., works	1.25		1.25		1.25	
Parathion, ethyl, drums, freight allowed	0.50	0.53	0.87		0.87	
Picric acid						
pure paste, 300 lb drums, dry basis, f.o.b. Charlotte, NC	2.00		2.00		2.00	
tech. paste, 300 lb drums, dry basis, f.o.b. Charlotte, NC	1.40		1.76		1.76	

Other major nitroaromatic compounds have experienced considerable growth and are projected to continue that trend. Table 22 summarizes past growth patterns and predictions of future growth. Markets for p-chloronitrobenzene (50% to p-nitrophenol) and p-nitrophenol (87% to parathions) are very dependent upon parathion consumption, which in turn is dependent upon cotton planting, and hence upon natural conditions. No growth was projected for p-nitroaniline, because most of the end-uses for the compound are under attack from other chemicals (Chemical Marketing Reporter, 1969). Inorganic pigments or other light-fast materials, for example, have virtually replaced pigments made from p-nitroaniline.

Table 22. Market Trends of Major Nitroaromatic Chemicals (Chemical Marketing Reporter, 1969, 1972, 1973 a, 1974 a, b)

Compound	Historical Growth	Projected Growth
p-Chloronitrobenzene	2% (1964-73)	3.5% (through 1978)
p-Nitroaniline	2.1% (1962-68)	no growth (through 1973)
Nitrobenzene	10.6% (1963-73)	7% (through 1978)
p-Nitrophenol	11.5% (1962-71)	3% (through 1976)
Parathions	10.5% (1962-72)	3% (through 1977)

B. Uses

1. Major Uses

Although some nitroaromatics are used as explosives (e.g., TNT), the major applications of nitroaromatic compounds are as chemical intermediates for dyes, pigments, pharmaceuticals, rubber chemicals, photographic chemicals, and agricultural chemicals. The applications of the major nitroaromatic compounds are listed with approximate volumes, when available, in Table 23. The large volume agricultural intermediates (50% of 1-chloro-4-nitrobenzene and p-nitrophenol) are somewhat unusual in that the nitro group is not used as a means of introducing an amine function, as is frequently the case with most of the other chemical intermediate applications.

Amination by reduction (Shreve, 1963) of the nitro functional group consumes considerable quantities of nitroaromatic intermediates (see Table 23). Reductive amination is a very old commercial process which can be traced back to 1847 when aniline was first manufactured commercially from nitrobenzene (Kouris and Northcott, 1963). The aromatic amines that are formed were initially consumed in the dye and pigment industry, but now a major portion of the production is consumed in the manufacture of such compounds as isocyanates (for production of polyurethanes) and rubber chemicals (aniline-based antioxidants and thiazole accelerators). The chemistry and synthesis approaches for the various chemicals derived from nitroaromatic compounds have been reviewed in the section on chemistry (Section I-B-1, p. 21). This section will discuss briefly some of the commercial reduction processes.

Reduction of nitrobenzene to aniline was first accomplished by the Béchamp process employing iron turnings and hydrochloric acid. The

Table 23. Uses of Major Nitroaromatic Chemicals

Compound	Application or Chemical Product	Approximate Volume of Nitroaromatic Compound Consumed, millions of pounds	% of Total	Reference
Nitrobenzene	Aniline	635,000	97	CMR (1974b)
	Other (chemical intermediate in dyes such as benzidine and explosives; Friedel-Crafts solvent; solvent in petroleum refining; mild oxidation agent	20,000	3	Matsuguma (1967a) Gilbert (1969) Lindner (1965) Lurie (1964) Olah and Cupas (1966) Nelson (1968)
2,4-(and 2,6)Dinitrotoluene	Toluene diisocyanate	~470,000	100	USITC (1959-73)
	Toluene diamine			Austin (1974)
	Produce 2,4-dinitrotoluene for smokeless powder	~50,000		Thirtle (1968) Small and Rosenblatt (1974)
2,4,6 Trinitrotoluene (TNT)	Major ingredient for composition B military explosive	432,000		Rosenblatt et al. (1973) Rinkenbach (1965)
	High explosive and propellant component			Lindner (1965) Dressler (1968)
	Chemical intermediate for 2,4,6-trinitrobenzoic acid			
1-Chloro-4-nitrobenzene	p-Nitrophenol	55	50	CMR (1974a)
	p-Nitroaniline	19	17	Industry sources
	Miscellaneous agricultural chemicals (excluding parathions)	11	10	
	Phenacetin	3	3	
	p-Aminophenol	5.5	5	
	Rubber chemicals	11	10	
	Other	5.5	5	
	Total	~110	100	
p-Nitrophenol and sodium salt	Ethyl and methyl parathions	52-87,000	87	CMR (1972)
	All other	8,13,000	13	Industry sources
	Total	60-100,000	100	
1-Chloro-2-nitrobenzene	Chemical intermediate for o-chloroaniline, o-nitroaniline, o-anisidine, o-phenetidine, o-aminophenol, and others	~60,000	100	Matsuguma (1967a) Wooster (1963) Kouris and Northcott (1963)

Table 23. Uses of Major Nitroaromatic Chemicals (Cont'd.)

Compound	Application or Chemical Product	Approximate Volume of Nitroaromatic Compound Consumed, millions of pounds	% of Total	Reference
	3,3'-Dichlorobenzidine	2,800	~5	Back calculation assuming 100% yield
Methyl parathion	Cotton insect poison	48,890		USITC (1959-73) CMR (1973a)
Trifluralin	Pesticide	25,000		Lawless et al. (1972) Plimmer (1970)
p-Nitrotoluene	5-Nitro-o-toluenesulfonic acid	~5,000		Matsuguma (1967a) and back calculations from production data for other compounds (assume 100% yield)
	Others (p-toluidine, other dye intermediates, TNT)	15-20,000		
	Total	20-25,000		
Parathion	Pesticide	15,259		USITC (1959-73)
o-Nitrophenol	Dye intermediate	10-15,000		Industry sources Matsuguma (1967b) Shreve (1963)
p-Nitroaniline	Rubber antioxidant	5,600	40	CMR (1969)
	Gasoline additives	2,800	20	
	Dyes and pigments	2,800	20	
	Pharmaceutical and veterinary use	980	7	
	Agricultural chemicals	420	3	
	Miscellaneous	1,400	10	
	Total	~14,000	100	
1,3-Dinitrobenzene	Intermediate for m-phenylenediamine	~12,000	~100	Industry sources Kouris and Northcott (1963) Thirtle (1968) Starr (1972) Russell (1971) Almerini (1966) Doe and Wood (1968)
	Possible TNT replacement			
	Cathodic material in batteries			
o-Nitrotoluene	o-Toluidine and other dye intermediates	10-12,000		Matsuguma (1967a)

Table 23. Uses of Major Nitroaromatic Chemicals (Cont'd)

Compound	Application or Chemical Product	Approximate Volume of Nitroaromatic Compound Consumed, millions of pounds	% of Total	Reference
4,4'-Dinitrostilbene-2,2'-disulfonic acid	Key intermediate for stilbene dyes	9,230		Schwander and Dominguez (1969)
5-Nitro-o-toluenesulfonic acid	4,4-Dinitrostilbine-2,2'-disulfonic acid (optical brightener intermediate)	7,955	~100	Schwander and Dominguez (1969)
1-Chloro-3-nitrobenzene	m-Chloroaniline, 2,2'-dichlorobenzidene, and other dye intermediates	7,908		Matsuguma (1967a)
1-Chloro-2,4-dinitrobenzene	Chemical intermediate for azo dyes, sulfur blacks, fungicides, rubber chemicals, and explosives (e.g. 2,4-dinitroaniline, 4-nitro-2-anisidine, 4-chloro-3-anisidine, 4-chloro-1,3-phenylenediamine, and picric acid)	6,626	100	Matsuguma (1967a) Wolfson (1967) Kouris and Northcott (1963)
	2,4-Dinitrophenol	1,100	16	Back calculation assuming 100% yield
1-Nitronaphthalene	α-Naphthylamine	6,290	100	Industry sources Treibl (1967)
o-Nitroaniline	Known as Azoic Diazo Compound 6 (C.I. 37025); used to prepare a few azo and anthraquinone dyes	6,000		Kouris and Northcott (1963)
m-Nitrobenzenesulfonic acid and sodium salt	Dye intermediate	3,654		Bannister and Olin (1965)
1,2-Dichloro-4-nitrobenzene	Intermediate for 2-Chloro-4-nitroaniline	3,000-3,600		Industry sources Wooster (1963)
2-sec-Butyl-4,6-dinitrophenol	Pesticide	3,000		Lawless et al. (1972)

Table 23. Uses of Major Nitroaromatic Chemicals (Cont'd)

Compound	Application or Chemical Product	Approximate Volume of Nitroaromatic Compound Consumed, millions of pounds	% of Total	Reference
2-Chloro-4-nitroaniline	Dye intermediate	2,500-3,000		Industry sources
o-Nitroanisole	Anisidine intermediate	2,500-3,000		Industry sources
p-Nitroanisole	Anisidine intermediate	750-1,500		Industry sources
2,4-Dinitrophenol	Chemical intermediate for sulfur dyes, azo dyes (2,4-diaminophenol, 4-nitro-2-aminophenol); photochemicals, pest control agents, wood preservatives, and explosives.	1,000		Matsuguma (1967 b)
	Polymerization inhibitor in styrene production			Coulter *et al.* (1969)
2-Bromo-4,6-dinitroaniline	Dye intermediate	944		Stenger and Atchison (1964)
m-Nitrobenzoic acid and sodium salt	Chemical intermediate for azo dyes	911		Duncker (1964)
2-Nitro-p-toluidine	Dye intermediate	864		Bannister and Olin (1965) Ehrich (1968) Johnson *et al.* (1963)
1,4-Dichloro-2-nitrobenzene	Mostly 2,5-dichloroaniline Other dyes (4-chloro-2-nitroaniline, 4,4'-dichloro-2-amidophenyl ether, 4-chloro-2-nitrophenol, and 4-chloro-2-nitroanisole)	700-800		Industry sources Matsuguma (1967a) Wooster (1963)
4-Chloro-3-nitrobenzenesulfonamide	Chemical intermediate	743		USITC (1959-73)
4-Chloro-2-nitrotoluene	Azo dye intermediate (e.g., 4-chloro-2-toluidine)	693		Matsuguma (1967a)
2,4-Dinitroaniline	Intermediate, toner, azo dye intermediate	>679		Kouris and Northcott (1963) Ehrich (1968) Matsuguma (1967a)

Table 23. Uses of Major Nitroaromatic Chemicals (Cont'd)

Compound	Application or Chemical Product	Approximate Volume of Nitroaromatic Compound Consumed, millions of pounds	% of Total	Reference
2,6-Dichloro-4-nitroaniline	Fungicide Dye intermediate	607		Cappellini and Stretch (1962) McMillan (1972)
2-Chloro-5-nitrobenzene-sulfonic acid and sodium salt	Pigment intermediate Synthesis of diarylamines	500-600		Industry sources Kehe (1965)
7-(and 8)Nitronaph[1,2]-oxadiazole-5-sulfonic acid	Chemical intermediate	551		USITC (1959-73)
3,5-Dinitrobenzoic acid	Reagent for identification of alcohols Explosives ingredient	∿500		Duncker (1964) Pristera et al. (1960)

oxidation of the iron to the ferrous or ferric ion results in reduction of the nitro group to an aromatic amine by the following equation:

$$RNO_2 + 3Fe + 4H_2O \xrightarrow{FeCl_2} RNH_2 + Fe(OH)_2 + FeO + Fe(OH)_3 + (H)$$

The Béchamp process may still be used in some small batch reactions, but has been replaced in most large scale reductions by catalytic hydrogenation.

Practically all nitroaromatic compounds can be reduced by catalytic hydrogenation using either vapor or liquid (usually alcoholic solution) phases (Shreve, 1963). However, hydrogenation is not selective enough for partial reductions of compounds containing more than one nitro group, and, in those cases where partial reduction is required, other reducing agents have to be used (see Section I-B-1). Perhaps a typical hydrogenation plant is the fluid-bed catalytic vapor phase hydrogenation plant operated by American Cyanamid at Willow Island, WV, which produces aniline from nitrobenzene (see Figure 14). The feedstock consists of nitrobenzene containing less than 10 ppm nitrothiophene. The nitrobenzene is vaporized, mixed with three times the theoretical amount of hydrogen, and passed over the catalyst (copper on silica). After cooling with condensation, the condensed aniline, aniline-water (water generated by the reaction), and excess hydrogen are separated. The crude aniline, which contains less than 0.5% nitrobenzene and about 5% water, is crudely distilled. The product is dehydrated and distilled to a purity of about 98%.

Reduction of nitroaromatic compounds under basic conditions results in the formation of hydrazobenzene derivatives, which are easily converted to benzidine derivatives (see Section I-B-1). Sizable quantities of nitroaromatic compounds are consumed at benzidine and benzidine-derivative production plants.

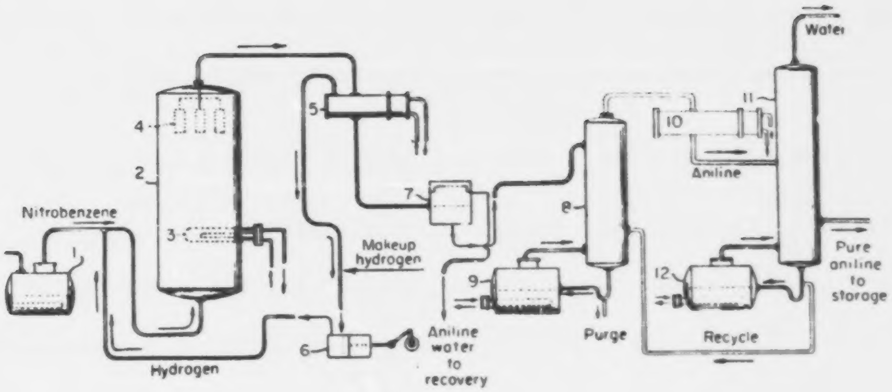

Figure 14. Continuous Fluid-Bed Vapor Phase Reduction of Nitrobenzene (Shreve, 1963)

1. Nitrobenzene vaporizer	7. Aniline-water settler and decanter
2. Reactor with fluidized catalyst bed	8. Crude aniline still
3. Cooling tubes	9. Reboiler for crude aniline still
4. Catalyst filters	10. Condenser
5. Product condenser	11. Aniline-finishing still
6. Hydrogen recycle compressor	12. Reboiler for aniline-finishing still

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Table 24 lists the manufacturers of some large volume aromatic amines that are derived from nitroaromatic compounds. Some other aromatic amines that have been produced by reduction, but still maintain a nitro substituent,have been discussed in Section I-B, p. 21).

2. Minor Uses

There are a sizable number of nitroaromatic compounds that are produced and consumed in small commercial quantities. Information on their uses is not plentiful or quantitative. The available information, which has been taken mostly from the Kirk-Othmer Encyclopedia of Chemical Technology, is tabulated in Table 25. As with the major nitroaromatic compounds, the major use of the small volume nitroaromatics is as chemical intermediates, with nitration used for introduction of amine functional groups.

Table 24. Large Volume Aromatic Amines Produced by Reduction of Nitroaromatic Compounds (SRI, 1975; Dean, 1971)

Nitroaromatic Compound	Aromatic Amine	Company	Location	Capacity (millions of pounds)
Nitrobenzene	Aniline	American Cyanamid	Bound Brook, NJ	60
			Willow Island, WV	50
		DuPont	Beaumont, TX	200
			Gibbstown, NJ	130
		First Mississippi	Pascagoula, MS	100
		Mobay	New Martinsville, WV	100
		Rubicon	Geismar, LA	55
2,4-(and 2,6-)Dinitrotoluene	Toluene diisocyanate (made from toluene-2,4-diamine)	Allied	Moundsville, WV	70
		BASF Wyandotte	Geismar, LA	40
		DuPont	Deepwater, NJ	170
		Mobay	New Martinsville, WV	100
			Cedar Bayou, TX	100
		Olin-General Tire	Ashtabula, OH	40
		Olin	Lake Charles, LA	90
		Rubicon	Geismar, LA	30
		Union Carbide	Institute, WV	55
	Toluene-2,4-diamine	Air Products	Pasadena, TX	
		American Cyanamid	Bound Brook, NJ	
		DuPont	Deepwater, NJ	
		GAF	Rensselaer, NY	
		Olin	Lake Charles, LA	
			Ashtabula, OH	
			Brandenburg, KY	
			Rochester, NY	
		Rubicon	Geismar, LA	
		Union Carbide	Institute and South Charleston, WV	

Table 25. Uses of Minor Nitroaromatic Chemicals

Chemical	Use	Reference
N-Acetyl-4,4'-dinitrodiphenylamine	Intermediate, precursor of 4,4'-diaminodiphenylamine	Thirtle (1968)
2-(p-Aminoanilino)-5-nitrobenzenesulfonic acid	Intermediate	USITC (1959-73)
2-Amino-5-nitrobenzenesulfonic acid	Intermediate	USITC (1959-73)
6-Amino-4-chloro-5-nitrophenol	Azo dye intermediate, antiamebic agent	Morse (1963) Elslager (1969)
2-Amino-4-chloro-5-nitrophenol	Intermediate for production of azo, acid, and mordant dyes	Morse (1963)
2-Amino-4-chloro-6-nitrophenol	Dye intermediate for mordant dyes	Morse (1963)
2-Amino-6-chloro-4-nitrophenol	Azo dye intermediate in production of mordant dyes	Morse (1963)
2-Amino-4,6-dinitrophenol (picramic acid)	Diazo base for azo dyes, explosive	Morse (1963)
2-Amino-5-nitrophenol	Dye intermediate for azo and oxidation dyes	Morse (1963)
4-Amino-2-nitrophenol	Dye intermediate, fur dye, hair dye (blond), Oxidation Base 25CCI 76555 (fur dye)	Morse (1963); Orton (1969); Markland (1966)
2-Amino-4-nitrophenol	Dye intermediate, hair dye (reddish)	Markland (1966)
4-Amino-5-nitrophenol	Hair dye	Tucker and Schwartz (1971)
6-Amino-4-nitro-1-phenol-2-sulfonic acid	Intermediate for azo dyes	Morse (1963)
4-Amino-6-nitro-1-phenol-2-sulfonic acid	Dye intermediate (Mordant Red 80(CI 26565))	Morse (1963)
2-Amino-6-nitro-1-phenol-4-sulfonic acid	Intermediate for azo dyes (wool and anodized aluminum)	Morse (1963)

Table 25 . Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
4-Amino-4'-nitro-2,2'-stilbenedisulfonic acid	Stilbene dye intermediate	Schwander and Dominguez (1969)
Ammonium picrate	Explosive	Rinkenbach (1965)
2-Bromo-6-chloro-4-nitroaniline	Intermediate	USITC (1959-73)
2-*sec*-Butyl-4,6-dinitrophenol alkanolamine salt ammonium salt isopropanolamine salt triethanolamine salt	Pesticides	SRI (1975)
2-*sec*-Butyl-4,6-dinitrophenyl-3,3-dimethylacrylate (Binapacryl)	Insecticide	USITC (1959-73)
N-Butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine (Benefin)	Pesticide	SRI (1975)
6-*tert*-Butyl-3-methyl-2,4-dinitroanisole (Musk ambrette)	Perfume material	Industrial sources
N-*sec*-Butyl-*p*-nitroaniline	Intermediate in preparation of N,N'-di-(*sec*-butyl)-*p*-phenylenediamine	Thirtle (1968)
1-*tert*-Butyl-3,4,5-trimethyl-2,6-dinitrobenzene (Musk tibetene)	Perfume material	Industrial sources
5-*tert*-Butyl-2,4,6-trinitro-*m*-xylene (Musk xylene)	Perfume material	Industrial sources
2-Chloro-3,5-dinitrobenzenesulfonic acid	Azo dye intermediate used to prepare 6-amino-4-nitro-1-phenol-2-sulfonic acid	Morse (1963)
2-Chloro-3,5-dinitrobenzotrifluoride	Used in preparation of 2-fluoro-3,5-dinitrobenzotrifluoride	Barbour *et al*. (1966)

Table 25 . Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
6-Chloro-2,4-dinitrophenol	Azo dye intermediate (used in commercial preparation of 2-amino-6-chloro-4-nitrophenol)	Morse (1963)
4-Chloro-2,6-dinitrophenol	Azo dye intermediate (used in commercial preparation of 2-amino-4-chloro-6-nitrophenol)	Morse (1963)
4-Chloro-2-nitroaniline	Dye intermediate	Wooster (1963)
4-Chloro-3-nitrobenzenesulfonic acid	Dye intermediate (commercial synthesis of 2-amino-1-phenol-4-sulfonic acid)	Morse (1963) Elliott and Bannister (1968)
4-Chloro-3-nitrobenzenesulfonyl chloride	Intermediate	USITC (1959-73)
2-Chloro-4-nitrobenzoic acid	Intermediate for dyes and medicinal chemicals, especially acridine derivatives	Duncker (1964)
2-Chloro-5-nitrobenzoic acid	Azo dye intermediate	Duncker (1964)
5-Chloro-2-nitrobenzoic acid	Dye intermediate	Duncker (1964)
2-Chloro-5-nitrobenzotrifluoride	Used in preparation of 2-fluoro-5-nitrobenzotrifluoride	Barbour *et al.* (1966)
4-Chloro-5-nitrobenzotrifluoride	Used in preparation of 4-fluoro-5-nitrobenzotrifluoride	Barbour *et al.* (1966)
o-(4-Chloro-3-nitrobenzoyl)benzoic acid	Intermediate	USITC (1959-73)
4-Chloro-6-nitro-1-phenol-2-sulfonic acid	Dye intermediate (synthesis for 6-amino-4-chloro-1-phenol-2-sulfonic acid)	Morse (1963)
6-Chloro-2-nitro-1-phenol-4-sulfonic acid	Dye intermediate (preparation of 2-amino-6-chloro-1-phenol-4-sulfonic acid)	Morse (1963)
2-Chloro-4-nitrotoluene	Dye intermediate	Matsuguma (1967a)

Table 25. Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
2-Chloro-6-nitrotoluene	Dye intermediate	Matsuguma (1967a)
4-Chloro-3-nitrotoluene	Intermediate	USITC (1959-73)
Diaminotrinitrobenzene	Spacecraft propellant	Industry sources
2-Diazo-4,6-dinitrophenol	Initiating explosive, especially in electric blasting caps and detonators	Matsuguma (1967b) Rinkenbach (1965)
2,6-Dibromo-4-nitroaniline	Pesticide	SRI (1975)
2,5-Dichloro-1-nitrobenzene	Dye intermediate	Matsuguma (1967a)
2,4-Dichloro-1-nitrobenzene	Intermediate (azo dyes, fungicides, rubber chemicals, and explosives)	Matsuguma (1967a)
Dichloronitrobenzoic acid, isometric mixture	Pesticide	SRI (1975)
2,5-Dichloro-3-nitrobenzoic acid	Intermediate for synthesis of amiben	Plimmer (1970)
4,6-Dichloro-2-nitrophenol	Dye intermediate (commercial preparation of 2-amino-4,6-dichlorophenol)	Morse (1963)
2',5-Dichloro-4'-nitrosalicylanilide (Niclosamide)	Antitapeworm chemotherapy Molluscacide	Mrozik (1967) Metcalf (1968)
2,4-Dichlorophenyl-4-nitrophenyl ether (Nitrofen)	Pesticide	SRI (1975)
2,6-Diiodo-4-nitrophenol	Used in dog hookworm therapy	Mrozik (1967)

Table 25 . Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
N,N-Dimethyl-*o*-nitroaniline	Intermediate for preparation of N,N-dimethyl-*o*-phenylenediamine	Thirtle (1968)
N,N-Dimethyl-*m*-nitroaniline	Intermediate (preparation of N,N-dimethyl-*m*-phenylenediamine)	Thirtle (1968)
4,6-Dinitro-2-aminophenol	Hair coloring (reddish)	Markland (1966)
Dinitroanilines	Herbicides	Plimmer (1970)
o-(2,4-Dinitroanilino)phenol	Intermediate	USITC (1959-73)
p-(2,4-Dinitroanilino)phenol	Intermediate	USITC (1959-73)
2,4-Dinitroanisole	Explosives ingredient	Pristera *et al*. (1960)
3',4-Dinitrobenzanilide	Intermediate	USITC (1959-73)
2,4-Dinitrobenzenesulfonic acid	Dye intermediate (preparation of 2,4-diaminobenzenesulfonic acid)	Thirtle (1968)
2,4-Dinitrobenzenesulfonic acid, sodium salt	Surface active agent	SRI (1975)
2,2'-Dinitrobenzidine	Dye intermediate for Mordant Yellow 21	Lurie (1964)
3,3'-Dinitrobenzidine	Formerly a dye intermediate for Sulfur Brown 13	Lurie (1964)
4,4'-Dinitrodiphenylamine	Preparation of 4,4'-diaminodiphenylamine	Thirtle (1968)
3,5-Dinitrobenzoyl chloride	Reagent for identifying alcohols	Duncker (1964)
4,6-Dinitro-*o*-*sec*-butylphenol ammonium salt triethanolamine salt	Pesticide	Mitchell (1961) SRI (1975)
Dinitro capryl phenyl crotonate	Pesticide	SRI (1975)

Table 25. Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
2,4-Dinitro-*o*-cresol	Herbicide	McNeil (1965)
4,6-Dinitro-*o*-cresol and sodium salt	Molluscacide Pesticide	Metcalf (1968) SRI (1975)
4,6-Dinitro-*o*-cyclohexylphenol	Pesticide, molluscacide	Metcalf (1968)
2,4-Dinitrodiazobenzene	Dye intermediate	Johnson *et al*. (1963)
4,4'-Dinitrodiphenylamine	Intermediate (4,4'-diaminodiphenylamine)	Thirtle (1968)
4,6-Dinitro-2-(1-methylheptyl)phenyl crotonate (Karathane)	Fungicide (against powdery mildew on some fruits, flowers, and shrubs)	Gearhart (1965)
4,6-Dinitro-2-methylphenol	Leather fungicide	Turner (1966)
2,6-Dinitro-1-phenol-4-sulfonic acid	Dye intermediate	Morse (1963)
Dinitroresorcinol	Explosive	Rinkenbach (1965)
2,4-Dinitroresorcinol	Explosive for detonators, caps, and ignitors	Rinkenbach (1965) Matsuguma (1967b)
2,4'-Dinitro-4-trifluoromethyldiphenyl ether	Pesticide	SRI (1975)
2,2',4,4',6,6'-Hexanitrodiphenylamine	Explosives ingredient Dye intermediate	Pristera *et al*. (1960) Matsuguma (1967a)
4-Hydroxy-3-nitrobenzenesulfonic acid	Dye intermediate	Elliott and Bannister (1968)
3-Hydroxy-3'-nitro-2-naphthanilide	Dye intermediate	SRI (1975)
N-(2-Hydroxy-5-nitrophenyl)glycerine	Hair dye (blond)	Markland (1966)
2-Iodo-3-nitrobenzoic acid	Plant growth regulator	Duncker (1964)
Lead 2,4-dinitroresorcinate	Explosive	Rinkenbach (1965)

Table 25 . Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
Lead picrate (trinitrophenolate)	Initiator (too dangerous for practical use because of very high sensitivity to impact)	Rinkenbach (1965)
Lead styphnate (2,4,6-trinitroresorcinate)	Initiating explosive (relatively poor)	Rinkenbach (1965)
2-(1-Methyl-*n*-heptyl)-4,6-dinitrophenyl crotonate (Dinocap)	Pesticide	SRI (1975)
3'-Nitroacetanilide	Intermediate	USITC (1975)
5-Nitro-4-amino-1,3-dimethylbenzene	Dye intermediate	Kouris and Northcott (1963)
N-Nitro-1-aminonaphthalene	Azo dye intermediate	Johnson *et al*. (1963)
4-Nitro-2-aminophenol	Azo dye intermediate	Morse (1963)
4-Nitroaniline-3-sulfonic acid (6-nitrometanilic acid)	Intermediate (ammonolysis to 4-nitro-*m*-phenylenediamine)	Thirtle (1968)
4-Nitro-3-anisidine	Azo dye intermediate	Matsuguma (1967a)
4-Nitro-*o*-anisidine	Azo dye intermediate	Matsuguma (1967a)
5-Nitro-*o*-anisidine	Azo dye intermediate	Matsuguma (1967a)
Nitrobenzaldehydes	Limited use in dye field	Deinet and DiBella (1964)
o-Nitrobenzaldehyde	Azo dye intermediate, indigo and its derivatives	Matsuguma (1967a)
o-Nitrobenzenesulfonyl chloride	Intermediate in manufacture of orthanilic acid	Gilbert (1969)
2-Nitrobenzidine	Formerly a dye intermediate	Lurie (1964)
3-Nitrobenzidine	Formerly a dye intermediate	Lurie (1964)

Table 25. Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
p-Nitrobenzoic acid	Manufacture of procaine, p-aminobenzoic acid, and esters of p-hydroxybenzoic acid	Duncker (1964)
p-Nitrobenzyl bromide	Reagent in qualitative organic analysis	Stenger and Atchison (1964)
6-Nitro-1-diazo-2-naphthol-4-sulfonic acid	Azo dye intermediate	Johnson et al. (1963)
4-Nitrodiphenylamine-2-sulfonic acid	Dye intermediate	Thirtle (1968)
Nitronaphthalenesulfonic acids	Intermediates in preparation of naphthylaminesulfonic acids	Treibl (1967)
p-Nitrophenacyl esters	Used to identify fatty acids	Elam (1965)
m-Nitrophenol	Dye production (anisidine)	Kouris and Northcott (1963)
6-Nitro-1-phenol-2,4-disulfonic acid	Azo dye intermediate	Morse (1963)
4'-(p-Nitrophenyl)acetophenone	Intermediate	USITC (1959-73)
2-Nitrophenylamine	Propellant component	Lindner (1965)
4-Nitrophenylarsonic acid	Control of histomoniasis (blackhead) in turkeys and chickens	Shor and Magee (1970)
2-Nitro-p-phenylenediamine	Hair dye (reddish, light brown)	Markland (1966)
4-Nitro-o-phenylenediamine	Hair dye (reddish, dark/medium brown)	Markland (1966)
5-Nitro-m-phenylenediamine	Hair coloring (reddish)	Markland (1966)
4-Nitro-m-phenylenediamine	Intermediate	Thirtle (1968)
N-(p-Nitrophenyl)glycine	Hair dye	Markland (1966)
m-Nitrophenylhydroxylamine	Dye intermediate in manufacture of 4-amino-2-nitrophenol	Morse (1963)

Table 25 . Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
Nitropyridines	Amebicides	Elslager (1969)
4-Nitropyrogallol	Preparation of benzenetetrol	Dressler (1968)
m-Nitrotoluene	Preparation of *m*-nitrobenzaldehyde, *m*-toluidine	Matsuguma (1967a)
3-Nitro-*p*-toluenesulfonic acid	Intermediate	USITC (1959-73)
5-Nitro-*o*-toluenesulfonic acid	Dye intermediate, stilbene dyes	Zweidler (1969)
Pentachloronitrobenzene	Pesticide	SRI (1975)
1,1,3,3,5-Pentamethyl-4,6-dinitroindan	Perfume material	SRI (1975)
Picramic acid, sodium salt	Photographic chemical	USITC (1959-73)
Picramic acid (2-amino-4,6-dinitrophenol)	Explosive, diazo base for azo dyes	Morse (1963)
1,2,4,5-Tetrachloronitrobenzene	Pesticide	SRI (1975)
Tetryl (N-methyl-N-nitro-2,4,6-trinitroaniline)	High explosive (booster charge)	Rinkenbach (1965)
3-Trifluoromethyl-4-nitrophenol	Lamprey larvicide	Metcalf (1968)
2,4,6-Trinitroaniline	(Explosive) Hair dye (reddish); in nitrobenzene used to extract cesium from fission product waste; used to extract potassium salt from sea water	Markland (1966) Davis (1964) McIlhenny (1967)
1,3,5-Trinitrobenzene	Explosive Preparation of 2,4,6-trinitroaniline Vulcanizing agent for natural rubber	Rinkenbach (1965) Kouris and Northcott (1963) Barnhart (1968)
2,4,6-Trinitrobenzoic acid	Synthesis of phloroglucinol	Dressler (1968)

Table 25. Uses of Minor Nitroaromatic Chemicals (Cont'd)

Chemical	Use	Reference
2,4,6-Trinitrochlorobenzene	Preparation of trinitroaniline (picramide)	Kouris and Northcott (1963)
2,4,6-Trinitrophenol (picric acid)	Dye intermediate, explosive, analytical reagent, germicide, fungicide, staining agent and tissue fixative, tanning agent, photochemical, pharmaceuticals, process material for oxidation and etching of iron, steel, and copper surfaces	Matsuguma (1967b) Morse (1963)
	Naphthalene picrate	Thiessen (1967)
2,4,6-Trinitroresorcinol (styphnic acid), lead salt	Initiating explosive	Matsuguma (1967b)

3. Possible Alternatives to Use

Chemical intermediate uses are the major application of the nitroaromatic compounds and, therefore, by varying the synthesis approach to the desired final product, alternatives to nitroaromatics may be possible. In many cases, the nitro functional group is used to introduce an amine group.

There are two major commercial ways of introducing amines: (1) amination by ammonolysis and (2) amination by reduction (Shreve, 1963). Reductive amination usually requires a nitro substituent, but ammonolysis can be used to substitute an amine group for a number of other functional groups. Ammonolysis can be broadly defined as "the cleavage of a bond by the addition of ammonia" (Wooster, 1963). The reaction is presently used to produce nitroanilines from chloronitrobenzenes (e.g.,*p*-nitroaniline from *p*-chloronitrobenzene) (see Figure 6, p. 26, for further examples). Substitution of $-NH_2$ for chlorines located *ortho* or *para* to nitro groups (or carboxylic groups) is possible with relatively mild (175°C, 530-580 psi) conditions because the chlorine atoms are "labilized" (Wooster, 1963) by the nitro group (see Section I-B-1 for mechanism). However, other ammonolysis processes which require more rigorous conditions have been commercially used to produce aromatic amines.

Aniline has been produced from chlorobenzene using ammonolysis and a copper catalyst. Because the chlorine has not been "labilized", a catalyst is required along with temperatures of 200 to 210°C and pressures of 850 to 950 psi. For the process to be economically competitive to nitration-reduction, the ammonolysis plant should be located near large-scale (therefore, inexpensive) production of chlorine and chlorinated products (Wooster, 1963). The crude product from the autoclave is a complex mixture containing aniline, ammonia, chlorobenzene, phenol, diphenylamine, and copper and ammonia compounds. Although a complex product results, the process has been adapted to continuous operation.

Ammonolysis may in many cases provide an alternative to the nitration-reduction approach to aromatic amines. The production of aniline from chlorobenzene and o-phenylenediamine from o-dichlorobenzene has reached commercial stages, although p-phenylenediamine from p-dichlorobenzene does not appear to be a technically satisfactory process yet (Wooster, 1963). Chloroanilines from bromochlorobenzenes are technically feasible because the bromine is more readily replaced than the chlorine. The application of ammonolysis to toluene and xylene derivatives will probably be dependent upon the reaction conditions. For example, p-chlorotoluene can be converted to p-chlorobenzonitrile rather than p-toluidine by vapor phase ammonolysis using the proper catalysts. Substitution of ammonolysis for nitration-reduction will be dependent upon catalyst and chemical engineering developments which will determine the economics of the alternative processes.

A variation of ammonolysis, commonly referred to as the Bucherer reaction, may also be an important alternative to nitration-reduction. The Bucherer reaction consists of the conversion of naphthols to naphthylamine derivatives, using sulfite catalysts. The suggested mechanism is depicted in Figure 15. The reaction is generally effective for replacement of naphthol and resorcinol, but not phenol, hydroxyl groups.

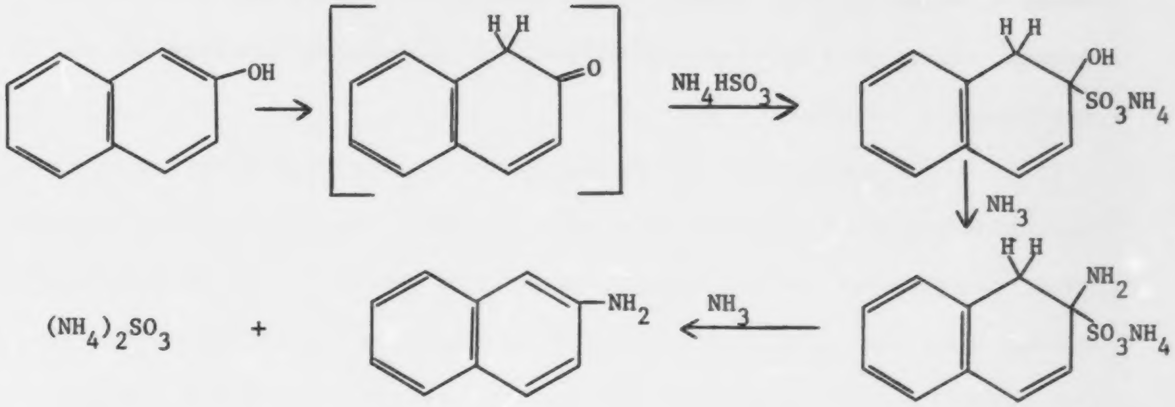


Figure 15. Mechanism of Bucherer Reaction (Wooster, 1963)

The Bucherer reaction occurs also between hydroxyl groups and primary amines; this has been used commercially for many years to produce N-phenyl-2-naphthylamine from aniline and β-naphthol. Up until about 1973, the corresponding α-compound, N-phenyl-1-naphthylamine, was produced by the reaction between the product of nitration-reduction of naphthene (1-naphthylamine) and aniline. However, N-phenyl-1-naphthylamine is now produced by the Bucherer reaction with α-naphthol and aniline. Although this has resulted in reduced necessity for the production of α-naphthylamine, the amine is still produced in significant quantities by nitration-reduction for other applications.

C. Environmental Contamination Potential

1. General

The major source of environmental release of nitroaromatic compounds appears to be from production plants and from by-product manufacturing plants. The release from these plants will depend upon the reaction processes, effluent treatment, and disposal procedures used, and these are likely to vary considerably for different compounds and at plants of different companies. Very little exact information on treatment and disposal procedures is available for individual plants, and the effluent air and water monitoring data are inadequate for quantitating release. For the vast majority of the numerous nitroaromatic compounds, the quantities of material released can only be estimated. However, from the available information, it can be concluded that the major potential source of contamination is from chemical plants and not from final product use. (An exception to this is contamination from the use of nitroaromatic pesticides).

2. From Production and Uses

It is frequently difficult to divide nitroaromatics into production and uses, since the use of one nitroaromatic compound may be the production of another. Many of these processes are carried out in aqueous media (e.g., hydrolysis of p-chloronitrobenzene to p-nitrophenol) or are worked up with water (e.g., water wash after nitration step or aqueous drawn tanks that are used to quench nitration reactions that are out of control). These water solutions can be a major source of environmental contamination if not properly treated. A number of nitroaromatic compounds have been detected in wastewater effluents from a number of chemical plants (see Table 31, p. 128). The compounds detected in effluents so far include nitrobenzene, chloronitrobenzenes, nitrophenols and -cresols, and nitrotoluenes. Similar compounds have been found in drinking water and raw river water.

No nitroaromatic compounds have been detected in air samples (Table 31, p. 138). This lack of detection is difficult to explain since many of the nitroaromatic compounds have appreciable vapor pressures and are probably released to the atmosphere during production and use, as well as transport, storage, and disposal.

Rosenblatt *et al*. (1973) and Small and Rosenblatt (1974) have conducted a detailed survey of nitroaromatic munitions wastes which included estimates of quantities released (calculated from average concentration and quantity of effluents) and estimates of concentrations to be expected downstream from the plants. These estimates are tabulated in Table 26. Only estimates of 2,4,6-trinitrotoluene (TNT) and dinitrotoluenes (DNT) have been reported but other nitroaromatic compounds are probably present. The effluents and treatment processes used are described in Section II-D-5, p. 108.

Release estimates for other commercial nitroaromatic compounds are not available, but they would be extremely useful in providing an assessment of environmental hazard.

3. From Transport and Storage

No information was available that would allow an intelligent estimate of losses of nitroaromatics during transport and storage. In general, the higher volume chemicals seem more likely to suffer larger losses due to spills and accidents than the smaller commercial products. It is probable that some losses do occur with all the chemicals, but the quantity lost is unknown.

4. From Disposal

Incineration, land burial, and, in the past, ocean dumping have been used to dispose of nitroaromatics. It is unlikely that incineration results in any significant release of nitroaromatics, but land burial may allow leaching into ground water or evaporation into the atmosphere if a proper site is not chosen. Ocean dumping of munition wastes is no longer practiced.

Table 26. Estimates of Trinitrotoluene and Dinitrotoluene from Army Munitions Plants (From Rosenblatt *et al*., 1973 and Small and Rosenblatt, 1974)

Plant Location	Effluent Discharge to	Compound	Quantity Released (lbs/day)	Maximum Concentration in Downstream Rivers or Water Supplies (mg/ℓ)
TNT Production				
Volunteer, TN	Tennessee R.	TNT	210	0.014 (Chattanooga)
		DNT	550	0.022 (Chattanooga)
Radford, VA	Stroubles Creek into New River	TNT	105	0.025 (Kanawha R.) 0.002 (Ohio R.)
		DNT	40	0.01 (Kanawha R.)
Joliet, IL	Illinois R.	TNT	61	0.006 (Peoria)
		DNT	530	0.030 (Peoria)
Load, Assemble, and Pack Plants				
Kingsport, TN	Holston R.	TNT	150	0.038 (Morristown)
Burlington, IA	Shink R. into Mississippi R.	TNT	1	< 0.001 (Keokuk)
Baraboo, WI		DNT	6	0.0006 (Muscoda)

5. Potential Inadvertent Production of Nitroaromatics in Other Industrial Processes as a By-Product

Nitration processes rarely produce pure isomers of a desired product. Any given process may produce a variety of undesired by-products which may be a major source of environmental contamination if not properly treated. With TNT, a major pollution problem was the "red water" generated in the Sellite process used to remove impurities. o-Chloronitrobenzene, a by-product of p-chloronitrobenzene, is another example of a by-product that has become an environmental contaminant (Council on Environmental Quality, 1971). Nitroaromatics may also be formed in non-nitration processes. For example, Knowles *et al*. (1974, 1975) have identified o-nitrophenols in smoked bacon. These compounds were postulated to have formed from the oxidation of the nitroso derivative.

6. Potential Inadvertent Production in the Environment

No information in the literature examined suggested that nitroaromatic compounds are formed in the environment. From chemical considerations, it would appear that these compounds could be produced: (1) by the oxidation of natural or man-made aromatic amines, and (2) by the reaction between NO_x in highly polluted air and aromatic hydrocarbons with activating substituents.

D. Current Handling Practices and Control Technology

1. Special Handling in Use

Toxic body levels of many of the nitroaromatic compounds can be reached by skin absorption, inhalation, or ingestion; considerable caution must be exercised in handling these materials. The following discussion is based upon safety data sheets developed for nitrobenzene, p-nitroaniline, and dinitrotoluene (Manufacturing Chemists Association, 1966 a, b, 1967) but is generally applicable to other nitroaromatics.

Nitroaromatic compounds should be handled only in well-ventilated areas, and air monitoring is recommended during handling of some of the more volatile compounds. Personnel should wear protective clothing and respirators where necessary, and food should not be consumed in the handling areas. With poly-nitro-compounds, explosions may occur if the compounds are heated, and, therefore, engineering controls are necessary to prevent localized heating (Bateman et al., 1974).

2. Methods for Transport and Storage

Liquid nitroaromatics, such as nitrobenzene, are shipped in drums, tank trucks, or tank cars. Nitrobenzene is classified by the Department of Transportation as a Poisonous Liquid, Class B, and as such, must be packed in specified containers when shipped by rail, water, or highway. There are also regulations regarding nitrobenzene loading, handling, and labeling.

Solids, such as 2,4-dinitrotoluene, may be shipped in fiber or metal drums. (The latter should be used if the product is melted before use). Shipping solid material in a molten state in tank cars or tank trucks is a common practice. With dinitrotoluene, the temperature of the unloading operations should be kept between 75° to 90°C, and localized overheating should be prevented. Pumps should not be used for unloading because of the hazard of explosion from heat generated by friction inside the pump.

Nitroaromatic compounds may be stored in shipping containers or in bulk storage containers. Storage tanks should be kept away from all sources of fire and excess heat. Outdoor storage is preferable, but if that is not possible, the storage tank should be equipped with a vent that terminates outdoors. If nitrobenzene is stored outdoors, precautions should be taken to prevent freezing, which could cause tank rupture. Dinitrotoluene storage tanks should be kept at temperatures less than 90°C and should be protected by deluge sprinkler systems.

Occasionally, tank trucks or cars and related equipment need to be repaired or cleaned; the tanks are drained, then washed with a hot, detergent wash (dinitrotoluene) or steamed (nitrobenzene and *p*-nitroaniline).

3. Disposal Methods

In general, it is recommended that all local, state, and federal regulations concerning waste disposal of nitroaromatics be determined and complied with (Manufacturing Chemists Association, 1966 a, b, 1967). Small amounts of nitroaromatics may be burned in an open field or in a properly designed chemical waste incinerator. With dinitrotoluene, care should be taken to ensure that no material goes to the incinerator in confined containers in order to prevent explosions. Forsten (1973) has reported that incineration of TNT has been successfully accomplished. Large quantities of liquid dinitrotoluene may be well diluted with fuel oil and burned safely in a liquid chemical incinerator. Nitroaromatic wastes may also be buried in a landfill set aside for toxic wastes. Such landfills should be located "where the water will not seep into underground water courses used as a source of drinking water, onto farmland or into streams and other bodies of water" (Manufacturing Chemists Association, 1967).

Waters from tank or spill clean ups may contain nitroaromatic wastes and may be treated in a variety of ways. Dinitrotoluene aqueous wastes should be cooled and settled to recover the bulk of the chemical for disposal by the methods described above. When adequate assimilative capacity is available, the remaining aqueous liquor may be discharged to a receiving stream or municipal sewage with regulatory approval. If permitted by regulatory authorities, nitrobenzen waste may be disposed of by dilution to less than 1% slurry and washed into a sewer connected to a municipal treatment plant.

Nitroaromatic munitions (e.g., TNT and tetryl) have been dumped at sea by scuttling old Liberty Ships (Hoffsommer and Rosen, 1972); since 1964 at least 18,342 tons of ammunition and explosives have been dumped (Council on Environmental Quality, 1970). In 1973, no explosives were disposed of by ocean dumping (Cox, 1975).

4. Accident Procedures

If a nitroaromatic chemical contacts the skin of a worker, the contaminated clothing should be removed and the affected area washed with soap and water. Ingestion of nitroaromatics should be treated by inducing vomiting, and gastric lavage should be performed as soon as possible. Individuals exposed to nitroaromatic vapors should be removed from exposure and kept under observation until a physician arrives. If the patient becomes cyanotic, oxygen may be administered.

Spills should be cleaned up immediately. If the material is a solid, it should be shoveled up, taking care to protect personnel with dust respirators and rubber gloves. The area should be promptly washed after major removal of the spilled material.

Fire fighting procedures for the nitroaromatics vary for different compounds. Nitrobenzene and p-nitroaniline fires can be extinguished with water, carbon dioxide, or chemical foam. Both compounds produce noxious fumes, but the combustion products of p-nitroaniline are particularly toxic and hazardous. Water is also effective with fires of unconfined dinitrotoluene, but with confined dinitrotoluene, no attempt should be made to fight the fire because of the explosion hazard. The surrounding area should be evacuated for protection of personnel and fire fighting capabilities should be supplied by remotely controlled systems.

Two case studies of explosions from a dinitrotoluene pipeline (Bateman _et al_., 1974) and a nitroaniline reactor (Vincent, 1971) have been reported. Both cases seem to have resulted from rather unique circumstances.

5. Current Control Technology

With the exception of the nitroaromatic compounds used as explosives, which are manufactured at government-owned contractor-operated plants (Forsten, 1973), very little information is available about air and water pollution control technology that is used with individual nitroaromatic compounds during manufacture or use. As noted in the section on disposal, wastes may be incinerated, buried in a landfill, sent to a municipal water treatment plant, etc., depending upon local, state, and federal regulations. Many of the nitroaromatic compounds are not very volatile and scrubbers are frequently used on nitration reactor vents (Process Research Inc., 1972), thus resulting in environmental release of nitroaromatic compounds into water effluents. However, many of the large volume nitroaromatic compounds (e.g. nitrobenzene, dinitrotoluene) have relatively high vapor pressure which could result in sizable vapor releases.

Considerable information is available on the water treatment procedures used with nitroaromatic munitions wastes. Although the wastes from these plants are atypical (the last nitration step for TNT uses 109% sulfuric acid and 98.5% nitric acid and the isomer purity requirements are much more stringent), the techniques may be somewhat similar to those used with other nitroaromatics.

TNT is the military explosive produced in the largest quantities. Water effluents from its production and use are unique in that they have even received common names. Following nitration, crude TNT is washed with water to remove acid. These washings, which are termed "yellow water", are returned to early stage nitrators, or incinerated if not recycled. Spent acid is sent to be recovered. A major pollution problem involved with TNT production is an effluent termed "red water", which is produced during the aqueous sodium sulfite washing step (Sellite) that is used to remove non-2,4,6-TNT isomers (about 5% of crude product). "Red water" contains approximately 25% solids - 9% organics, 10.6% sodium sulfite, 0.6% sodium sulfate, 3.5% sodium nitrite, and 1.7% sodium nitrate (Rosenblatt, 1973). In the past, "red water" was disposed of by dumping into a convenient stream (Forsten, 1973). Presently, it is concentrated by evaporation and either sold to paper plants for its sulfur content or incinerated (Rosenblatt et al., 1973). Recently, scientists at the Picatinny Arsenal have suggested that ammonium sulfite be substituted for sodium sulfite (Anon., 1975 c) in order that the washing can be mixed with spent acid and collected in a spent acid recovery system. (Sulfur is recovered as sulfur dioxide and reused, ammonia is converted to nitrogen, and production is increased by 8%).

"Pink water" is generated both in manufacturing plants and load, assemble, and pack plants (LAP's). In manufacturing plants "pink water," so named because of its pink color under neutral or basic conditions especially when exposed to sunlight, can be generated in:(1) Mahon fog filter (anti-air pollution systems) (Siele and Ribaudo, 1971), (2) nitrator fume scrubber discharges, (3) "red water" distillates (from concentration step), (4) finishing building hood scrubber and wash-down effluents, and (5) possibly spent acid recovery wastes (Rosenblatt et al., 1973). These effluents contain 2,4,6-TNT and other isomers, as well as less-nitrated isomers (e.g., dinitrotoluene) and other by-products. Carbon adsorption was for a long while considered economically unattractive because the carbon could not be regenerated because of the explosion hazard. Now carbon adsorption treatment of "pink water" may be used with a toluene leach regeneration step with the toluene then used as a feedstock. "Pink water" also results from shell washout operations in LAP's; it contains mostly pure 2,4,6-TNT. The general practice for disposing of TNT wastes from LAP's is to use evaporation ponds, although some plants use activated charcoal (Rosenblatt _et al_., 1973). Nay (1972) has studied the biodegradability of TNT wastes with activated sludge pilot plants; he concluded that TNT oxidized more slowly than it was transferred to the biomass; therefore, contact stabilization should be used if biological systems were used to treat TNT. It is unknown whether biological treatment processes are currently being used.

Tetryl (2,4,6-trinitrophenylmethylnitramine) is no longer being manufactured, but, when it was produced at Joliet, Illinois, wastewaters from the process were routed to two parallel drainage ditches (Small and Rosenblatt, 1974). Wastewaters from trinitroresorcinol production are routed to lagoons and, once every few years, the sludge is removed to a landfill (Small and Rosenblatt, 1974).

E. Monitoring and Analysis

1. Analytical Methods

Since a variety of functional groups are found on the commercially important nitroaromatic compounds, numerous analytical approaches are possible. Gas chromatography appears to be by far the most popular technique, but liquid and plasma chromatography and mass spectrometry have also been frequently used. The first two subsections of this section will review a variety of methods that have been used both for commercial product analysis and for trace analysis of explosive and pesticide nitroaromatics. The third section will review methods that have been used for trace analysis of non-pesticide or non-explosive chemicals and/or have actually been used for environmental monitoring.

a. Explosives

A large number of nitroaromatic compounds find application as explosives. Because of military and security considerations and the fact that TNT (trinitrotoluene) production processes cause many environmental problems, analytical methods for these nitroaromatic compounds are well developed. Most of the compounds that fall into the category of explosive materials are polynitroaromatic hydrocarbons, although other functional groups, such as chlorine or hydroxyl groups, are not uncommon. The methods and their applications have been summarized in Table 27. Many of the methods are probably applicable only to analysis of the explosive formulations (e.g., spot test, infrared and nuclear magnetic resonance spectrometry), but many of the techniques have been used or have great potential for environmental monitoring. Electron capture detectors respond similarly for nitroaromatics and chlorinated aromatics; since the electron capture detector is one of the most sensitive detectors known, very small concentrations of nitroaromatic compounds can be detected by gas chromatography or by thin layer chromatography with electron capture detection. Hoffsommer and coworkers (1972) were able to detect compounds at parts per trillion levels in sea water; a similar procedure was capable of measuring 5 ppt in soil (Hoffsommer, 1975). The strong absorption of nitroaromatics in the ultraviolet wavelength region makes high pressure liquid chromatography with UV detection quite attractive for trace analysis; Doali and Juhasz (1974) suggest sensitivities well into the nanogram range. A relatively new analytical development, plasma chromatography, appears to be applicable to detection of nitroaromatics in water or in air samples when only picogram quantities are isolated (Karasek and Denney, 1974; Wernlund, 1973). Mass spectrometers, alone or combined with gas chromatographs, provide a very specific technique for determining nitroaromatics at very low concentrations (ppb - Wall and Gage, 1973; Karasek, 1974).

b. Pesticides

The methods used to analyze the nitroaromatic pesticides are similar to the techniques for the explosives. Gas chromatographic techniques for dinitrophenols and p-nitrophenol (parathion degradation product) are widely used, especially with electron capture detection. Since this review is not as concerned with pesticides as it is with other commercial nitroaromatics, these techniques are only briefly reviewed in Table 28, and monitoring data for pesticides have not been covered in Section II-E-2.

Table 27. Analytical Methods for Nitroaromatic Explosives

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Colorimetry	MacKay *et al.* (1958)	TNT in air	Bubbling of air through peroxide-free ethylene-glycol monoethyl ether	Violet color obtained by adding alkali to the TNT in the ether solution, sensitive to 2 μg/ml of ether (used 2-8 ml of ether in sampler when sampling approximately 1.5 cubic feet of air).
Spot test	Coldwell (1959)	Explosives	Organic nitrates and nitramines are dissolved in acetone and reacted with diphenylamine when U.V. induced	Method distinguished between nitrates and nitramines, could differentiate between TNT, tetryl, picrite, and picric acid. Sensitivity not reported.
Spot test	Sawicki and Stanley (1960)	Polynitroaromatic compounds		Blue or green color was developed with cyclopentadiene methylene group (fluorine) and alkaline. Positive for aromatic compounds with two or three electron-attracting groups in the *meta* position. Identification limit dinitrobenzene (0.1 μg), 2,4-dinitrochlorobenzene (0.05 μg), 2,4-dinitrotoluene (0.02 μg), 2,4-dinitroaniline (0.01 μg), and picric acid (0.05 μg).
Color test	Hackett and Clark (1960)	Explosives including TNT, tetryl, picric acid, and some mononitrotoluenes	Sample dissolved in ethanol, reduced to nitroso compound, color developed with pentacyanoaminoferrate	Test was used for rapid identifications in an explosives laboratory. Limits of detection were from 10 - 100 μg.
Infrared spectroscopy	Pristera *et al.* (1960)	Explosives and explosives ingredients		Compilation of infrared spectrograms of 68 compounds, mainly for qualitative use.
Infrared spectroscopy	Kite (1961)	Red water wastes from munitions plant	Extraction with MEK/butanol (80/20). Separation by column chromatography	Detection limits not reported.
Gas chromatography	Parsons *et al.* (1961)	Products of nitration of toluene		
Paper chromatography	Colman (1962)	14 Substituted trinitrobenzenes in 10 partition systems	Spotted on paper impregnated with formamide or heavy mineral oil	R_F's were determined for tetryl, picric acid, and picramide among others with various solvent systems. Results simplify selection of solvent system for separation of compounds in a mixture.

Table 27. Analytical Methods for Nitroaromatic Explosives (Cont'd)

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Thin layer chromatography	Yasuda (1964)	Crude trinitrotoluene		Two dimensional TLC was used to separate and identify TNT impurities. Silica gel G/zinc TLC plates were used. Spots were developed by p-diethylaminobenaldehyde (0.25%) and HCℓ (0.25 N).
	Yasuda (1970)	Tetryl (N-methyl-N,2,4,6-tetranitroaniline) and related compounds		Two dimensional TLC was used to separate and identify components of production grade tetryl and thermal decomposition product of tetryl (see above). Detection limit 0.5 to 1.0 μg.
Spot test	Amas and Yallop (1966)	Dinitro-and trinitroaromatics in industrial blasting explosives	Dissolve 5 -10 mg sample in acetone alcohol solution	Test using color developed with tetramethyl ammonium hydroxide was developed for forensic use. Limits of identification were 4 μg for m-dinitrobenzene (color not specified), 2 μg for 2,4-dinitrotoluene (blue), and 1 μg for α-trinitrotoluene (dark red).
Gas chromatography with flame ionization detection	Rowe (1966)	2,4,6-Trinitrotoluene in castable explosives		
Gas chromatography with thermal conductivity detection	Gehring and Shirk (1967)	Trinitrotoluene and dinitrotoluene isomers in crude and refined TNT		The method required high purity external standards and frequent instrument calibration. The lowest detectable amount is 0.02%, requires high-purity isomers for preparation of internal standards (Dalton et al., 1970).
Nuclear magnetic resonance spectrometry	Gehring (1970)	Impurities in crude TNT from the trinitration step, red oil exudate and extracts thereof		The purpose of the work was to define TNT nitration and purification processes. Limit of detection was 0.03%.
Gas chromatography with flame ionization detection	Dalton et al. (1970)	Organic phases of the continuous TNT process (mono-, di-, and trinitrotoluenes)	None, or ether extraction if the organics were in the acid phase	The need for frequent instrument calibration was avoided by using predetermined flame ionization detector responses to calculate relative percentages. Lowest concentration report 0.01%.

Table 27. Analytical Methods for Nitroaromatic Explosives (Cont'd)

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Thin layer chromatography	Kohlbeck et al. (1970)	TNT nitrator samples and TNT product		Two dimensional TLC separations were performed on samples made under varying conditions for the purpose of improving the continuous TNT process.
Thin layer chromatography and gas chromatography with electron capture detection	Hoffsommer (1970)	10 Nitro compounds, including TNT		Quantitative analysis of nitro compounds in micro- to picogram range. 1,3,5-trinitrobenzene was used as a standard. The detector is very sensitive; 1 x 10^{-6} g of TNB overloaded it.
Gas chromatography with electron capture detection	Hoffsommer and Rosen (1971, 1972)	TNT, RDX (not a nitroaromatic), and tetryl in sea water	Benzene extraction of sea water	Method capable of detecting TNT, RDX, and tetryl at 2, 5, and 20 parts per trillion, respectively, was used to monitor ocean dumping areas (no explosives were found in the samples).
Thin layer chromatography and gas chromatography with electron capture detection	Hoffsommer et al. (1972)	TNT, RDX, tetryl, and ammonium perchlorate in sea water, sediment, and ocean floor fauna	Sea water-benzene extraction. Sediment and fauna-benzene extraction, cleanup on TLC	Detection levels were as above but somewhat less sensitive with analysis of marine fauna (47 - 740 ppt). No explosives were found in the samples.
Gas chromatography with electron capture detection	Hoffsommer and Rosen (1973)	TNT, RDX, and tetryl in sea water		The method was used to determine progress of hydrolysis of these compounds in sea water.
Thin layer chromatography of charge-transfer complex formed with amines	Parihar et al. (1967)	2,4,6-Trinitrochlorobenzene, 1,3,5-trinitrobenzene		TLC plates were impregnated with various amines. The complex that formed was chromatographed. Procedure was able to resolve up to 2-3 µg.
Thin layer chromatography and spectrophotometry	Parihar et al. (1971)	6 Binary mixtures containing tetryl, TNT, picramide, etc.		Use of charge-transfer complexes of the compounds allow much better resolution. Up to 1.5 µg could be measured.
Gas chromatography - flame ionization detection (slight modification of Dalton et al., 1970)	Nay (1972) Nay et al. (1974)	TNT waste waters	Ether extraction	The method did not detect soluble nitroaromatic salts from the sellite process, or hexanitrodibenzyl.

Table 27. Analytical Methods for Nitroaromatic Explosives (Cont'd)

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Colorimetric measurements	"	TNT waste waters	Dilution where necessary	Color of waste waters was used as an indication of nitrobody concentration; a potassium chloroplatinate color standard was used.
Colorimetric measurements	"	TNT in waste waters	Filtration	α-TNT by Silas-Mason method (Standard Methods of Water Analysis, American Public Health Association, 1965 and 1971) was determined by measuring at 425 mμ the color of the complex between α-TNT and diethylaminoethanol; TNT was report as mg/ℓ (concentration range 1 - 50 mg/ℓ).
High speed liquid chromatography	Spano et al. (1972)	Aqueous effluents from TNT finishing processes	Water sample placed directly on XAD-2 resin	The work was done to study α-TNT wash waters as they were exposed to sunlight and neutralized. The concentration of pure α-TNT in the comparison sample was 100 ppm.
Negative ion mass spectrometry	Yinon et al. (1972)	TNT in trace quantities		Method was considered for possible use in TNT detector. Negative ion mass spectrometry was more specific than positive (focus on NO_2^- peak). At 20°C and equilibrium the TNT concentration would be 1 part in 10^6, which is well within the detection range. Mass spectrometer leak detectors have been reported to be capable of measuring picogram (10^{-12}) quantities.
Thin layer chromatography with ethylene diamine spot development	Chandler et al. (1972a)	TNT by-products from nitration vessel	Dilution with water and acetone	Method is capable of measuring all major oxidation products from continuous TNT nitration process. No detection limit reported.
High pressure liquid chromatography using methylene chloride solvent	Chandler et al. (1972b)	Hexanitrobibenzyl (HNBB) and 3-methyl-2',4,4',6,6'-pentanitrodiphenylmethane (MPDM) in TNT		In samples from continuous TNT process, HNBB varied from 0.1 - 0.5% and MDPM from 0.1 - 0.3%. Maximum concentrations found in batch process were 0.06% MDPM and 0.01% HNBB. Structures of MDPM and HNBB were assigned from NMR and IR spectra and TLC retention times.

Table 27. Analytical Methods for Nitroaromatic Explosives (Cont'd)

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Thin layer chromatography and gas chromatography with electron capture detection	Burlinson et al. (1973)	TNT and photoproducts in water	Benzene extraction	TLC methods indicated 8 photodecomposition of TNT in irradiated aqueous solutions of TNT (pink water). Sample initially contained 600 ppm TNT.
Liquid chromatography with ultraviolet absorbance and differential refractometer detection	Walsh et al. (1973)	Standard nitrotoluene solutions and TNT waste water	Water samples were used directly	TNT waste waters were studied for waste abatement purposes. The range of TNT measurements was 1 - 100 ppm. Quantities of less than 1 ppm were easily detectable.
Plasma chromatography	Wernlund (1973)	TNT in river water		Selective detection of nanogram and lower quantities of TNT in river water (Karasek and Denney, 1974).
Portable vapor detection systems (tested sensitivity and specificity)	Wall and Gage (1973)	TNT	Direct sampling of atmosphere	
Ion Mobility Spectrometer (version of plasma chromatography)				Ni^{63} emits beta particles which ionize molecules. The rate of migration in an electric field is characteristic of the molecule. TNT was not measured.
Bioluminescent Sensor System				Marine microorganisms luminesce when exposed to vapors of certain compounds. Light change is measured. Threshold concentration level in air for TNT was 30 ppb. False detections due to other compounds or changes in humidity were noted (poor specificity). Very portable system.
Mass Spectrometer				Portable quadrupole mass spectrometer with three stage membrane separator. Threshold level for TNT 25 ppb. Most specific method.
Model 58 Explosive Detector				Consists of a membrane and an electron capture detector. Threshold concentration for TNT was 0.2 ppb (most sensitive of all instruments tested). Specificity is imparted by different recovery times following detection. At elevated temperatures required for TNT, recovery times for different compounds are very similar. Therefore, the method is not very specific.

Table 27. Analytical Methods for Nitroaromatic Explosives (Cont'd)

Technique	Reference	Type of Sample	Isolation and/or Cleanup Method	Results and Comments
Electron capture detector	Cline *et al*. (1974)	TNT	Direct sample of atmosphere	Two sample streams. One passes through a decomposition chamber which allows differentiation between explosive (thermally unstable) and other background vapors.
Plasma chromatography or gas chromatography using plasma chromatograph detector	Karasek and Denney (1974)	TNT in air	Direct injection - no preconcentration	Method provided rapid detection and identification of picogram quantities of TNT and related compounds. Identification is provided by characteristic positive and negative mobility spectra.
High speed liquid chromatography (U.V. and differential refractometer detection)	Doali and Juhasz (1974)	Mixtures of explosives		The method could separate TNT and tetryl, a mixture of toluene, *p*-nitrotoluene, dinitrotoluene, and trinitrotoluene, etc. Sensitive well into the nanogram range with U.V. detector.
Automated 2 channel colorimetric analysis	Hess *et al*. (1975)	Nitro compounds (esp. trinitrotoluene and related compounds)	Water sample	One channel measures the color of nitro compounds with strong base (Meisenheimer complexes); the response here is attributable to the total of all nitro compounds present. In the other channel, 15% potassium hydroxide hydrolyzes nitro groups prior to reaction with a color reagent; the method is selective for trinitro compounds and is sensitive to 1 ppm TNT (reproducibility at 1 ppm is ± 10%).
Gas chromatography (flame ionization detection) and colorimetric method	Hess *et al*. (1975)	TNT manufacturing water effluent	GC samples extracted with benzene	GC method operating range 1 - 80 ppm. Authors compared the two techniques and concluded that the automated colorimetric technique is more useful for routine work but is slightly less accurate than the GC method.

Table 28. Methods Used for Analysis of Nitroaromatic Pesticides and Related Compounds

Technique	Reference	Type of Sample	Isolation Method	Results and Comments
Paper chromatography	Karlog (1957)	Parathion and *p*-nitrophenol in organic tissue material	A solvent extract of an acid extract was purified by column chromatography	It was recommended that the spots should contain 5 - 100 μg of *p*-nitrophenol. Quantitation of *p*-nitrophenol was determined by a U.V. spectrophotometer.
Paper chromatographic separation plus quantitative determinations by other methods	Erne (1958)	Parathion and *p*-nitrophenol in biological materials	Solvent extractions of acidified sample; alumina column cleanup	*p*-Nitrophenol was determined spectrophotometrically. The determination was sensitive to about 1 μg/g.
Comparison of methods: Colorimetry (1) Polarography (2) Microcoulometric gas chromatography (3)	Klein and Gajan (1961)	Pentachloronitrobenzene in vegetables	Ethanol petroleum ether extract, used as is for (2) and purified by column chromatography for (1) and (3)	(1) could measure down to 0.1 ppm (± 0.008 ppm); (2) down to 0.02 ppm (∓ 0.008 ppm); and (3) down to 0.1 ppm.
Gas chromatography with electron capture detection	Carey (1963)	Tetrachloronitroanisole (TCNA) on vegetables and grains	Benzene extract, column chromatography cleanup	Low concentration analyzed 0.013 ppm.
Gas-liquid chromatography with electron capture detector	Duggan *et al.* (1966)	Pentachloronitrobenzene and 1,2,4,5-tetrachloro-3-nitrobenzene in total diet composite samples	---	Sensitive to 0.001 ppm.
Oscillopolarography combined with thin layer chromatography	Hearth *et al.* (1968)	Parathion, paraoxon, and *p*-nitrophenol in processed peaches	Methylene chloride extraction	Accurate in the 0.5 - 2.0 ppm range
Gas chromatography with electron capture and flame ionization detectors	Hrivnak and Stota (1968)	Dinitrophenols and other substituted phenols	---	Use of polar polyester type columns prevented tailing (usually a problem with phenols) and allowed separation. With the electron capture detector, only a few nanograms were necessary for analysis.
Gas-liquid chromatography - thermal conductivity detection	Clifford and Watkins (1968)	Mixture of dinitroalkyl phenols	GLC with DEGA/phosphoric acid on Chromosorb W or G	Results indicate that DEGA modified with 0.4% phosphoric acid is effective in separating dinitroalkyl phenols. Limit of detection not reported.

Table 28. Methods Used for Analysis of Nitroaromatic Pesticides and Related Compounds (Cont'd)

Technique	Reference	Type of Sample	Isolation Method	Results and Comments
Thin layer (adsorption and reversed-phase) and gas-liquid chromatography	Clifford et al. (1969)	Homologous series of substituted dinitrophenols	---	---
Gas chromatography	Clifford and Watkins (1970)	Nitrophenols and nitroanisole	---	---
Gas chromatography with electron capture detection	Cranmer (1970)	p-Nitrophenol (PNP) in urine	Sample is hydrolyzed and PNP is extracted	Method is sensitive to 50 ppb for p-nitrophenol in urine. The trimethylsilyl ether derivative is made in the GC column by injecting PNP with hexamethyldisilazane.
High speed liquid chromatography with polarographic detector	Koen et al. (1970)	Mixture of parathion, methyl parathion, and p-nitrophenol	---	Concentrations of 10^{-8} mol/ℓ can be determined with a 2% standard deviation. Polarograph detector requires that the solvent system have a high conductivity.
Gas chromatography with electron capture or alkali flame ionization detection	Newsom and Mitchell (1972)	N,N-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine (dinitramine) in soil forage, and crops	Methanol extraction, methylene chloride partitioning, Florisil column cleanup	Sensitive down to 0.01 ppm.
Gas chromatography with electron capture detection	Shafik et al. (1973) Bradway and Shafik (1973)	Nitrophenols in urine	Hydrolyze sample, extract ethyl ether, cleanup on silica gel column	Limits of detection 0.01 - 0.05 ppm.
Gas chromatography with electron capture detection	Allen and Sills (1974)	3-Trifluoromethyl-4-nitrophenol (TFM) in fish	Hexane-ether extraction, partitioning, and methyl ether derivatization	Suitable recoveries at 0.01 - 2.00 μg/g fish muscle.
Automated gas-liquid chromatography with electron capture detection	De Vos et al. (1974)	Pentachloronitrobenzene tetrachloronitrobenzene, and 2,6-dichloro-4-nitroaniline in lettuce	Extraction with ethyl acetate - no cleanup	Extracts were diluted with hexane and analyzed automatically. The system was reportedly suitable for screening large series of sample with accuracy equal to that of manual analysis; 1-3 ppm added to lettuce at extraction stage were recovered 100 ± 5%.

Table 28. Methods Used for Analysis of Nitroaromatic Pesticides and Related Compounds (Cont'd)

Technique	Reference	Type of Sample	Isolation Method	Results and Comments
Gas chromatography with electron capture detector and flame photometer	Sherma and Shafik (1975)	Parathions in pesticide multiresidues in air	Methylene chloride extraction from ethylene glycol trapping solvent, then fractionation on silica gel column	In multiresidue method developed for National Air Monitoring Program, fractions from silica gel fractionation were gas chromatographed. The phosphate compounds were analyzed by flame photometry; the others, by electron capture method. For parathion, 50 and 207 ng added to 100 mℓ of ethylene glycol were recovered 93 and 90%, respectively; 40 and 162 ng of methyl parathion, 97 and 87%, respectively.
Gas chromatography with electron capture detection	Olson *et al.* (1975)	Dinitramine in fish	Extraction, Florisil cleanup	Limit of detection 0.01 mg/ℓ.
Gas chromatography with flame ionization detection	Klus and Kuhn (1975)	Nitrophenols in various samples	Acid-base partitioning, ether extraction, methylation with diazomethane	Easily measures 100 µg of various nitrophenols.

c. Miscellaneous Nitroaromatic Analytical Methods and Monitoring Studies

The analytical methods reviewed in this section have been applied more frequently in actual environmental monitoring situations; they are summarized in Table 30. Gas chromatography with flame ionization and/or electron capture (Zielinski *et al.*, 1967 b, uses both for qualitative purposes) has been quite popular for laboratory studies, but its application to monitoring has been infrequent. The relationship between chemical structure and electron capture sensitivity was studied by Zielinski *et al.* (1967 a). Their results, summarized in Table 29, demonstrate that most nitroaromatics exhibit large responses

Table 29. Relative Electron Capture Sensitivities of Nitroaromatic Compounds (Zielinski *et al.*, 1967 a)

Compound Group	Compound	Relative Sensitivity
Chlorobenzenes	*p*-Dichlorobenzene	1.88
Chloronitrobenzenes	2,3-Dichloronitrobenzene	2.34
	m-Chloronitrobenzene	2.21
	2,5-Dichloronitrobenzene	2.12
	2,4,5-Trichloronitrobenzene	2.10
	o-Chloronitrobenzene	1.66
	3,4-Dichloronitrobenzene	1.13
	2,4-Dichloronitrobenzene	1.11
	p-Chloronitrobenzene	1.00
Fluoronitrobenzenes	*o*-Fluoronitrobenzene	0.74
	p-Fluoronitrobenzene	0.685
	m-Fluoronitrobenzene	0.206
Dinitrobenzenes	*m*-Dinitrobenzene	1.63
	o-Dinitrobenzene	1.29
Nitroanilines	*o*-Nitroaniline	0.302
	m-Nitroaniline	0.260
Miscellaneous	*o*-Bromonitrobenzene	1.22
	2-Nitro-4-chloroaniline	0.214

Table 30. Miscellaneous Nitroaromatic Analytical and Monitoring Techniques

Technique	References	Type of Sample	Isolation and Cleanup Method	Results and Comments
Carbon filter chloroform extracts (CCE) and infrared analysis	Middleton and Lichtenberg (1960)	o-Chloronitrobenzene in the Mississippi River	CCE cleaned up chromatographically	Several thousand gallons pass through the sampler in 10 days. o-chloronitrobenzene was detected 105 and 1020 miles above the mouth of the Mississippi River.
Colorimetric method with air sampler	Hands (1960)	Nitrobenzene in air	Concentration in air sampler containing cellusolve	Zinc amalgam and hydrochloric acid were used to reduce the aniline which was then diazotized with α-naphthol salt. With a 6-liter sample, concentrations of 0.5 to 2 ppm v/v could be determined.
Midget impinger cold trap collection - U.V. analysis	Linch and Charsha (1960)	Nitrobenzene in air	Cold trap impinger was used for collection	Collection efficiencies for 1 and 5 ppm of nitrobenzene in air were 85% to 95% at -21°F and -116°F.
Gas chromatography with flame ionization (FI) detection	Habboush and Norman (1962)	Mixtures of isomers of disubstituted benzenes	---	The column packing materials that were capable of separating ortho, meta, and para isomers of nitrotoluenes, fluoronitrobenzenes, chloronitrobenzenes, bromonitrobenzenes, and nitroanisoles were reported.
Gas chromatography with FI	Selucky et al., (1967)	Nitrobenzene in air of nitrobenzene plant	Celite 545 concentration tubes were used	With the concentration tube, 10 mg of nitrobenzene per m^3 of air could be measured.

Table 30. Miscellaneous Nitroaromatic Analytical and Monitoring Techniques (Cont'd)

Technique	References	Type of Sample	Isolation and Cleanup Method	Results and Comments
Gas chromatography with electron capture (EC) detection	Zielinski *et al.*, (1967a)	Chloro-, bromo-, nitrobenzenes	---	Relationship between structure and sensitivity in electron capture analysis was determined.
Gas chromatography with dual EC-FI	Zielinski *et al.*, (1967b)	Chloronitrobenzene isomers	---	The ratio of the electron capture/flame ionization response was used for qualitative purposes. No detection limit noted.
Thin-layer chromatography (UV light development of spots)	Berei and Vasaros (1967)	Nitrobenzene and chloronitrobenzene isomers	---	*o*, *m*, and *p*-chloronitrobenzene could be separated.
Gas chromatography	Habboush and Tameesh (1970)	Fluoro-, chloro-, and bromonitrobenzenes; nitroanisoles; and nitrotoluenes	---	Retention times on various packing materials were presented.
Preparative gas chromatography and identification by IR and mass spectrometry	Friloux (1971)	Nitrobenzene, chloronitrobenzene, and dinitrotoluene in New Orleans finished water	Carbon chloroform extract (CCE)	No concentrations were noted.
Same as above	U.S. EPA (1972)	Number of nitroaromatics	Same as above	Because recoveries were not determined, quantitation was not possible.
Colorimetric Method	Tiwari and Pande (1972)	*m*-dinitrobenzene, 2,4,6-trinitrobenzene in nitrophenol 1-chloro-2,4-dinitrobenzene	---	Sensitive for 100-600 μg

Table 30. Miscellaneous Nitroaromatic Analytical and Monitoring Techniques (Cont'd)

Technique	References	Type of Sample	Isolation and Cleanup Method	Results and Comments
Colorimetric method	Kurenko (1972)	*o*-, *p*-nitrotoluene in air	Preconcentration by absorbers	Capable of detecting concentrations of 0.75 mg/m^3
Gas chromatograph-mass spectrometry	Burnham *et al.*, (1972)	*p*-nitrophenol and 4,6-dinitro-2-aminophenol in water	Preconcentration by macroreticular resin bed (XAD resins)	Recovery for *p*-nitrophenol was 100% at 0.2 ppm; for 4,6-dinitro-2-aminophenol recovery was 43% at 0.4 ppm. Procedure allowed detection of aromatic hydrocarbons down to 1 ppb.
Gas chromatograph-mass spectrometry	Webb *et al.*, (1973)	Industrial water effluents	Solvent extraction	20ℓ samples allowed apparent detection limits of 0.1 μg/ℓ while with 1 ℓ samples the detection limits were 2 μg/ℓ.
Plasma chromatography	Karasek and Kane (1974)	Halonitrobenzenes	---	Sample size was on the order of 10^{-7} to 10^{-8} g. Isomers could be identified by mobility spectra.
Hollow fiber probe with mass spectrometry	Westover *et al.*, (1974)	Nitrobenzene in air or water samples	---	Capable of continuous monitoring at ppm levels. Limit of detection ~1 ppm (methanol) to 10 ppb (chloroform) in water.
Gas chromatography with FI detection	Austern *et al.*, (1975)	Nitrobenzene in wastewater	Extraction in Freon	Minimum detectable quantity of nitrobenzene was 0.7 ng. Recovery from spiked raw and treated wastewaters was 100% at 0.317 mg/l for nitrobenzene.

with electron capture detectors. (Minimum detectable quantity with electron capture detectors = 10^{-13} g, Karasek, 1975.).

Techniques for monitoring nitroaromatics in air (Hands, 1960; Linch and Charsha, 1960; Selucky et al., 1967; and Westover et al., 1974) seem more suitable to occupational or effluent monitoring applications than to ambient analyses, since most of the techniques are sensitive only in the ppm range. None of the techniques noted in Table 30 have actually been used for ambient air monitoring. Because of the strong electron-capturing characteristics of most nitroaromatics, both gas chromatography with electron capture detection or plasma chromatography, which is also dependent upon electron capture, could be used for ambient air monitoring (e.g., see TNT detection in air, Karasek, 1974, and Karasek and Denney, 1974), especially if some preconcentration step were used (see Sherma and Shafik, 1975).

In contrast to the lack of data in ambient air monitoring, nitroaromatic compounds have been detected in raw river water and finished drinking water (Middleton and Lichtenberg, 1960; Friloux, 1971; U.S. EPA, 1972). Unfortunately, all of these researchers have used a carbon chloroform extraction (CCE) preconcentration step. Although this technique makes qualitative analysis easier (larger sample), it precludes quantitative analysis unless recovery studies are undertaken. In a study conducted on the lower Mississippi River (U.S. EPA, 1972), quantitation was not attempted; therefore, the following section (II-E-2) does not report concentrations. Burnham et al. (1972) have developed an XAD resin preconcentration step for drinking water that would allow quantitation (they used GC-MS), but so far this procedure has not been used extensively in field surveys.

With industrial wastewaters, the concentrations of contaminants is usually much higher, so smaller samples can be used. The most comprehensive study of wastewaters (Webb et al., 1973) used solvent extraction of a 1 or 20 liter sample, combined with GC-MS, to give a detection limit of 0.1 μg/ℓ for the larger sample.

In summary, there are a number of analytical methods that are specific and sensitive enough for trace analysis of nitroaromatics in ambient environmental samples. However, as will be noted in the following section, application of these techniques in monitoring studies has been infrequent.

2. Monitoring Studies

The available monitoring data on nitroaromatic compounds are summarized in Table 31. Ambient monitoring (including monitoring of drinking water) is presented in the top portion of the table, while effluent monitoring is in the lower part of the table. The lack of air monitoring data in the literature available for this report is very noticeable. It is difficult to attribute this lack to a particular cause, since many of the nitroaromatic compounds have appreciable vapor pressures and are probably released to the atmosphere in sizable quantities. The available water monitoring data is more extensive than the air monitoring data, but, considering the number of nitroaromatic compounds, it would seem to be far from adequate. Only six compounds have been detected at ambient levels, and none of the studies have provided quantitative data. Whether or not other nitroaromatic compounds are present in river or drinking water is unknown. However, there have been a number of monitoring studies that have had the capability to detect nitroaromatic compounds that have reported that detectable amounts were not present. These studies are summarized in Table 32. Perhaps the

Table 31. Ambient or Effluent Monitoring of Nitroaromatic Compounds

Reference	Analytical Method	Monitoring Site and Type of Sample	Chemical	Concentration (ppm)
Middleton and Lichtenberg (1960)	Carbon chloroform extract (CCE) with infrared spectrometry	Cape Girardeau, MO (1020 miles from mouth of Mississippi River)	o-Chloronitrobenzene	0.004 - 0.037
		New Orleans (105 miles from mouth)	same	0.001 - 0.002
Friloux (1971)	CCE	New Orleans drinking water	Chloronitrobenzene Dinitrobenzene Nitrobenzene	
Borodin and Kuchinskaya (1971)	Unspecified	Drinking water for the city of Tomsk, USSR	Nitrobenzene	
U.S. EPA (1972)	CCE	Carrollton drinking water plant, New Orleans	m-Chloronitrobenzene Nitrobenzene 2,6-Dinitrobenzene	
		Jefferson Parish #2, raw water, New Orleans	Nitrobenzene Nitroanisole*	
		Rubicon Chem. (nitrobenzene producer - 75 mill. lbs capacity)	Nitrobenzene	
U.S. EPA (1975)	---	Drinking water	4,6-Dinitro-2-aminophenol m-Chloronitrobenzene 2,6-Dinitrotoluene Nitrobenzene	
Golubeva (1957)	Colorimetric	Sewage of a petroleum refinery	Nitrobenzene	0.2 - 0.3
Kite (1961)	Solvent extraction, column chromatography, infrared spectrometry	Picatinny Arsenal, raw red water wastes, Dover, NJ	2,4-Dinitrotoluene-5-sulfonic acid 2,4-Dinitrotoluene-3-sulfonic acid 2,4-Dinitrotoluene 3,5-Dinitrobenzenesulfonic acid	
Jenkins and Hawkes (1963)	Unspecified	Fison's Pest Control Ltd., waste water, Harston, Cambridge, U.K.	Dinitro-o-cresol	

Table 31. Ambient or Effluent Monitoring of Nitroaromatic Compounds (Cont'd)

Reference	Analytical Method	Monitoring Site and Type of Sample	Chemical	Concentration (ppm)
Karelin et al. (1964)	Unspecified	Kuibyshev petroleum processing plant	Nitrobenzene	
Kurmeier (1964)	Unspecified	TNT manufacturing plant waste-water effluent	Dinitrotoluene Nitrotoluene	
Papov (1965 a, b)	Unspecified	Water effluent from sulfur dye production	2,4-Dinitro-1-chlorobenzene	
Trifunovic et al. (1971)	Unspecified	Water effluent from parathion production	p-Nitrophenol	
Nay (1972)	Gas chromatography-flame ionization	Radford TNT Plant, water effluent, Radford, VA	2-Nitrotoluene	0.32 - 16
			4-Nitrotoluene	0.12 - 9.2
			2,4-Dinitrotoluene	trace - 39
			2,6-Dinitrotoluene	3.39 - 56.3
			2,4,6-Trinitrotoluene (TNT)	101 - 143
			Trinitrobenzoic acid	0.80
Walsh et al. (1973)	Liquid chromatography	TNT finishing plant wastewater	2,4-Dinitrotoluene TNT	
Zetkin et al. (1973)	Unspecified	m-Chloronitrobenzene production wastewaters	m-, o-, p-Chloronitrobenzene	1.5 - 1.8 g/ℓ
Webb et al. (1973)	Solvent extraction of water effluent, GC-MS	Specialty chemical plant	4,6-Dinitro-o-cresol	18
		Explosives (DNT) plant	2,4-Dinitrotoluene	190
		" " " raw waste	2,6-Dinitrotoluene	150
		" " " pond effluent	" "	0.02
		TNT plant raw effluent	" "	0.68
		Explosives (DNT) plant	3,4-Dinitrotoluene	40
		Chemical companies lagoon after steam stripping	Nitrobenzene	0.11
		" "	2-Nitro-p-cresol	9.3
		" "	o-Nitrophenol	1.4
		Paper mill's five-day lagoon	o-Nitrotoluene	
		TNT plant's raw effluent	" "	0.15
		DNT plant's raw effluent	" "	7.8
		" "	m-Nitrotoluene	
		Chemical company's lagoon after steam stripping	p-Nitrotoluene	0.04
		DNT plant's raw effluent	" "	8.8
		TNT plant's raw effluent	2,4,6-Trinitrotoluene	0.7

* Compound was dropped from 1 Sept., 1975 List of Organic Compounds Identified in Drinking Water (U.S. EPA, 1975)

Table 32. Monitoring Studies Reporting No Detectable Quantity of Nitroaromatic Compounds

Reference	Analytical Method	Compounds the Analytical Method Could Detect	Monitoring Site and Type of Sample	Sensitivity of the Method
Kleopfer and Fairless (1972)	CCE concentration, GC-FI, MS, IR, NMR		Tap water	---
Burnham *et al.* (1972)	XAD resin concentration, GC-MS, GC	*p*-Nitrophenol 4,6-Dinitro-2-aminophenol	Well water Ames, IA	ppb range
Hoffsommer and Rosen (1971, 1972)	Solvent extraction, GC-EC	2,4,6-Trinitrotoluene (TNT), methyl-2,4,6-trinitrophenylnitramine (tetryl)	Sea water, 200 miles off coast of Florida, 45 miles west of San Francisco	ppb-ppt range
Hoffsommer *et al.* (1972)	"	"	Sea water, sediment, and ocean floor fauna; 85 miles west of Flattery, WA, 172 miles south-southwest of Charleston, SC	"
U.S. EPA (1974)	CCE	Method detected a number of nitroaromatics in earlier study (U.S. EPA, 1972)	Drinking water in New Orleans area	---

most significant study was the 1974 duplication (U.S. EPA, 1974) of an earlier study of New Orleans drinking water (U.S. EPA, 1972). The early study had detected several nitroaromatics, while the later study reported no nitroaromatic compounds. In summary, a number of nitroaromatic compounds have been detected in river and drinking water and in various effluents, but the available information is so sparse that it is difficult to determine whether nitroaromatic compounds are widespread environmental contaminants.

III. Health and Environmental Effects

A. Environmental Effects

1. Persistence

a. Biological Degradation, Organisms and Products

The biological transformation and particularly the microbial transformation of nitroaromatic compounds has received a fair amount of attention. The interest has arisen in recent years primarily because nitroaromatics are increasingly being used as inhibitory agents (e.g., pest control agents) or in the synthesis of inhibitory agents. The existence of naturally occurring biological nitro compounds (e.g., chloramphenicol, β-nitropropionic acid, etc.) (Ehrlich et al., 1948; Carter and McChesney, 1949; Hirata et al., 1954) suggests the possible existence of organisms able to decompose nitroaromatic compounds (Cain, 1958; Gundersen and Jensen, 1956).

An extensive review of the literature has revealed that the environmental fate related information is available for the following groups of compounds: nitrobenzenes and chloronitrobenzenes, nitrobenzoic acids, nitrophenols and related compounds, nitrotoluenes, and nitroanilines. Information is reviewed below for each of these categories.

(i) Nitrobenzenes and Chloronitrobenzenes

A number of researchers have examined the biodegradability of unsubstituted and halogen-substituted nitrobenzenes. A summary of the conditions employed in the biodegradation studies by various investigators is presented in Table 33. In one of the earlier studies, Alexander and Lustigman (1966), while studying the effect of chemical structure on the microbial degradation of substituted benzenes, found that nitrobenzenes were quite

Table 33. Summary of the Degradation Studies with Unsubstituted and Halogen Substituted Nitrobenzenes

Reference	Test Chemicals	Conc. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Alexander and Lustigman, 1966	Nitrobenzene, *o*, *m*, and *p*-dinitrobenzene	5-10 mg/ℓ	Soil (Niagara silt loam)	64 days	Loss of UV absorbancy
Bringmann and Kuehn, 1971	1,3,5-trinitrobenzene, *m*-dinitrobenzene, nitrobenzene	118-146 mg/ℓ	Combined action of *Azotobacter agilis*, and microorganisms in activated sludge	-	Estimation of nitro-reduced metabolites
Chambers *et al*., 1963	Nitrobenzene, *m*, and *p*-dinitrobenzene, 1,3,5-trinitrobenzene	100 mg/ℓ	Microorganisms in soil, compost, or mud from a catalytic cracking plant waste lagoon, adapted to degrade phenol	170-210 min	Oxygen consumption in Warburg
Malaney, 1960	Nitrobenzene	500 mg/ℓ	Aniline-acclimated activated sludge	8 days	Oxygen consumption in Warburg
Moore, 1949	Nitrobenzene	0.1% v/v	Two *Nocardia* sp., enriched from soil on pyridine	-	Growth on nitrobenzene as sole source of carbon, nitrogen, and energy
Villanueva, 1960	*o*, *m*, and *p*-dinitrobenzene, 1,3,5-trinitrobenzene	250 mg/ℓ	Pure culture of *Nocardia V*.	16 days	Growth

Table 33. Summary of the Degradation Studies with Unsubstituted and Halogen Substituted Nitrobenzenes (Cont'd)

Reference	Test Chemicals	Conc. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Bielaszczyk *et al*., 1967	*p*-chloronitrobenzene and 2,4-dinitrochlorobenzene	1. Stationary conditions: 100 mg/ℓ *p*-nitro 20 mg/ℓ dinitro 2. Continuous flow conditions: 9.2 mg/ℓ *p*-nitro and 1.7 mg/ℓ dinitro (concentration reduced by half after 3 days) 3. Two aeration columns: 92 mg/ℓ *p*-nitro and 17 mg/ℓ dinitro	*Arthrobacter simplex*, *Fusartub* sp., *Trichoderma viride*, *Spreptomyces coelicolor*, isolated from soil and industrial waste containing nitrochlorocompounds	8-13 days	Estimation of nitro and amino groups
Ludzack and Ettinger, 1963	*o*-chloronitrobenzene	21.1 mg/ℓ	Settled sewage added weekly	175 days	CO_2 production

difficult to degrade; both mono- and di-substituted (o, m, or p) nitrobenzenes persisted for more than 64 days (the criterion of persistence was the unchanged U.V. absorbancy [due to intact benzene ring] of the solution). The assay technique employed in this study required the use of a small amount of soil inoculum and precluded the addition of growth factors and supplemental organic compounds in order to minimize interferences. The recalcitrant nature of the nitro-substituted benzenes revealed by this test could thus be due to the unsuitability of the test conditions. The biological resistance of di- and trinitro-substituted benzene (o-, m-, and p-dinitrobenzene, and 1,3,5-trinitrobenzene) is, however, also suggested from the fact that these compounds failed to serve as sole carbon or nitrogen source for growth of Nocardia V (Villanueva, 1960). The culture of Nocardia V was able to reduce one nitro group of p-dinitrobenzene to an amino group resulting in the formation of p-nitroaniline; the enzyme responsible for this reaction has been extracted and purified from a Nocardia culture by Villanueva (1960, 1964). The selection of Nocardia V for studying utilization of nitro compounds was based on the reported evidence that some species of genus Nocardia are able to utilize a variety of aromatic compounds for growth (Bergey's Manual of Determinative Bacteriology, 1948; Moore, 1949; Cain, 1958). Azim and Mohyuddin (1957) have reported that nitrobenzene was not utilized as a source of nitrogen by Azotobacter vinelandii, an organism which fixes atmospheric nitrogen. The authors attributed this to the inability of nitrobenzene-N to be reduced to NH_3.

The number of chemicals entering the environment is enormous. When a synthetic chemical enters the environment, one or a group of indigenous populations possessing requisite enzymes or which can adaptively synthesize necessary enzymes frequently multiply and make use of the introduced substrate. It is likely that requisite acclimation may also occur when an organism in the environment comes in contact with a chemical which has the same basic configuration as the chemical to be degraded. With this view in mind, a number of researchers have determined whether a culture highly adapted to a compound representing a basic chemical configuration similar to nitrobenzenes is capable of degrading nitro-substituted benzenes. Malaney (1960) examined the ability of an aniline-acclimated activated sludge microflora to oxidize nitrobenzene. They found that under their experimental conditions, the endogenous oxygen uptake rate exceeded the oxygen uptake in the presence of the test chemical. This lead the author to conclude that nitrobenzene under the above conditions is poorly oxidized or not oxidized at all. A decrease in endogenous oxygen uptake due to the presence of nitrobenzene could also be due to its inhibitory action on oxidative enzymes. The ability of phenol-adapted bacteria to degrade nitrobenzene, *m*- and *p*-dinitrobenzene and 1,3,5-trinitrobenzene, has been examined by Chambers *et al*. (1963). Microorganisms present in soil compost, or mud from a catalytic cracking plant waste lagoon, were subjected to preliminary adaptation on phenol by techniques such as soil percolation, activated sludge aeration, primary enrichment in flask, or enrichment in batch-type fermenters. Further adaptation was carried out by subculturing the microorganisms periodically on mineral salts medium containing phenol as the only source of carbon. Cell suspensions adapted in this manner exhibited very low levels of oxidative activity on nitro substituted benzene compounds, particularly on the mono nitro-substituted benzene; in that case, the endogenous level of oxygen consumption was higher than that observed in the presence of nitrobenzene as was observed by Malaney (1960) with aniline-acclimated activated sludge. The resistance of the nitrobenzenes to degradation seemed to decrease as the number of nitro groups on the benzene ring increased (Table 34).

Table 34. Oxidation of Nitro-Substituted Benzenes by Phenol-Adapted Culture (Chambers *et al*., 1963)

Compound	Test Time (min)	Oxygen Consumed		Ratio of Endogenous to Test Compound
		Endogenous (cell alone)	Cells & Test Compound	
Nitrobenzene	170	53	32	>Endog.
m-Dinitrobenzene	210	57	102	1.8
p-Dinitrobenzene	180	65	97	1.5
1,3,5-Trinitrobenzene	180	65	127	2.0

Contrary to the reports that nitrobenzenes are difficult to degrade, Moore (1949) reported the isolation of two *Nocardia* sp. from soil which used nitrobenzene as a sole source of carbon, nitrogen, and energy. The organisms employed by Moore (1949) in studying degradation of nitrobenzene were isolated from pyridine enrichment cultures.

The biological removal of 1,3,5-trinitrobenzene, *m*-dinitrobenzene and nitrobenzene in a two-stage model waste water purifier has been reported by Brigmann and Kuehn (1971). The system consisted of an aerator (1st stage) which was inoculated with *Azotobacter agilis*, and 2nd stage overflow basin which was inoculated with activated sludge. The authors found practically complete removal of the mono nitro compounds in the aeration stage. In the case of trinitrobenzene, complete removal was not achieved until after passage through the 2nd stage. No information concerning the nature of the metabolites formed or the extent of removal due to adsorption on the biological material was revealed in this study.

Nitrochloro-substituted benzene compounds of commercial significance which have been examined for their biodegradability include p- and o-nitrochlorobenzene, and dinitrochlorobenzene. Bielaszczyk et al. (1967) have reported isolation of microorganisms which reduced nitrochloro-compounds to the corresponding amino compounds. The organisms were: Arthrobacter simplex, Streptomyces coelicolor, Fusarium sp. and Trichoderma viridis which were isolated from soil, and a species of Arthrobacter simplex isolated from industrial waste containing nitrochloro-compounds. A mixture of the above microorganisms was considerably more effective than individual microorganisms in reducing nitro-compounds. Under continuous flow conditions involving feeding, aeration, settling and reflux, and containing a mixture of p-nitrochlorobenzene and 2,4-dinitrochlorobenzene inoculated with Arthrobacter simplex, the reduction of the nitrochlorobenzene reached 61-70% and dinitrochlorobenzene was reduced quantitatively. When two aeration columns were used, one with Arthrobacter, the other with Arthrobacter, Fusarium, Trichoderma and Streptomyces, a reduction up to 90% of nitrochlorocompounds after 10 days was observed. The reduction of nitrochlorobenzene gave rise to p-chloroaniline and some undefined products. After reduction of dinitrochlorobenzene, nitroanilines and some unidentified products were formed.

The fate of o-chloronitrobenzene in Ohio River water supplemented by weekly addition of settled sewage to provide nitrogen, trace nutrients, and new organisms, has been studied by Ludzack and Ettinger (1963).

Biodegradation was assessed from the evolution of CO_2. The authors found no degradation of nitrochlorobenzene after incubation periods as long as 175 days.

In the case of o-chloronitrobenzene, the environmental stability determination can also be based to some extent on the available monitoring data. This compound has been shown to travel long distances in surface water as evidenced by the fact that the reduction in concentration of the compound observed along 1450 Km of the Mississippi River could be totally explained by dilution factors (Kramer, 1965). These findings suggested that o-chloronitrobenzene remained unaltered, at least during the time period required for transport down 1450 river kilometers.

In summary, the available information regarding the environmental fate of nitro- and chloronitro-substituted benzenes tends to suggest that these compounds will not degrade at appreciable rates by microorganisms in the environment. The nitro-group of certain nitro-substituted benzenes has been shown to be reduced by microorganisms, and it is likely that such modification may occur in the environment also; however, the one compound (o-chloronitrobenzene) that has been monitored in the environment does not appear to undergo such reduction. Other pathways of degradation of these compounds in the environment have not been well studied. The resistance of nitrobenzenes to extensive microbial degradation is supported by the fact that very infrequently have researchers succeeded in enriching microbial populations from natural mixed cultures, which will utilize nitro-substituted benzenes as the sole source of carbon or nitrogen.

(ii) Nitrobenzoic Acids

The microbial metabolism of nitrobenzoic acid received much attention after the suggestion that some of these compounds may be intermediates in the reduction of nitrates by green plants and microorganisms. The studies dealing with biodegradation of substituted and unsubstituted nitrobenzoic acids have been summarized in Table 35.

(a) Mono Nitrobenzoic Acids

Microbial Degradation

From the inability of phenol adapted bacteria to oxidize o-, m- and p-nitrobenzoic acid, Chambers et al. (1963) concluded that nitrobenzoic acids are resistant to biodegradation. Unlike the results of Chambers et al. (1963), a number of researchers have reported the isolation of microorganisms capable of growing on nitrobenzoates which may be suggestive of the biodegradable nature of these chemicals. Cain (1958) isolated *Nocardia erythropolis* from p-nitrobenzoate enrichment and *N. opaca* from o-nitrobenzoate enrichment, both of which were capable of utilizing the respective nitrobenzoic acids as sole sources of carbon, nitrogen, and energy. The enrichment cultures were set up using garden soil, or water from polluted streams as the source of natural mixed cultures. The presence of m-isomer inhibited the oxidation of o- and p-nitrobenzoic acid. However, m-nitrobenzoic acid was found to be oxidized to some extent by organisms grown on either p-nitrobenzoate or o-nitrobenzoate. None of the isolates were able to utilize hydroxy substituted (2-, 3-position) p-nitrobenzoic acid. After continued efforts, Cain and his coworker (Cartwright and Cain, 1959 a) succeeded in isolating a *Nocardia* sp. (referred to as *Nocardia* M1), which was capable of metabolizing the meta-isomer of nitrobenzoic acid. Since nitrobenzoates supported good growth only under alkaline conditions, Cain (1958) suggested that the substrate was assimilated in the dissociated form (pK values

Table 35. Summary of the Studies Dealing with Biodegradation of Substituted and Unsubstituted Nitrobenzoic Acids

Reference	Test Chemical	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Alexander and Lustigman, 1966	*o*-, *m*-, and *p*-Nitrobenzoic acid	5-10 ppm	Soil (Niagara silt loam)	64 days	Loss of UV absorbancy
Cain, 1958	*o*-, *m*-, and *p*-Nitrobenzoic acid 2,4-Dinitrobenzoic acid 3-Hydroxy-4-nitrobenzoic acid	0.5-10 ppm	Species of *Nocardia* and *Pseudomonas* isolated from soil and polluted stream water	7-16 days	Growth; formation of NH_3, arylamine, etc.
Cain *et al*., 1968	*p*-Nitrobenzoate, 2-chloro-, 2-bromo-, 2-iodo-, 3-fluoro-4-nitrobenzoate	2-10μmoles/3 mℓ	*Nocardia erythropolis* grown on *p*-nitrobenzoates	Up to 2 hours	Oxygen uptake, assay of metabolites
Cartwright and Cain, 1959a	*o*-, *m*-, and *p*-Nitrobenzoic acid	1000 ppm	*Nocardia opaca* *Nocardia M1* *Nocardia erythropolis*	--	Uptake of oxygen, output of CO_2, and production of either ammonia or nitrite
Cartwright and Cain, 1959b	*o*-, *p*-Nitrobenzoic acid	4.0 mM	*Nocardia erythropolis*, *N. opaca*, *Nocardia M1*, and *Pseudomonas fluorescens* isolated from soil	24 hours	Reduction to the corresponding arylamine
Chambers *et al*., 1963	*o*-, *m*-, *p*-Nitrobenzoic acid, 2,5-dinitrobenzoic acid, 3,4-dinitrobenzoic acid, 2,4,6-trinitrobenzoic acid	60-100 ppm	Microorganisms in soil, compost, or mud from catalytic cracking plant waste lagoon, adapted to degrade phenol	3-3.5 hours	Oxygen uptake
Durham, 1958	*p*-Nitrobenzoic acid	Growth: 0.1-0.2%; oxygen Uptake: 4 mM	*Pseudomonas fluorescens*	Growth: 20 hours; Oxygen uptake: 2 hours	Growth, oxygen uptake

Table 35. Summary of the Studies Dealing with Biodegradation of Substituted and Unsubstituted Nitrobenzoic Acids (Cont'd)

Reference	Test Chemical	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Germanier and Wuhrmann, 1963 (Abstract)	*p*-, and *o*-Nitrobenzoic acid	mM Concns. (actual concn. not specified)	*Pseudomonas* strain isolated from soil	4 Days	Growth and formation of NH_4^+, NO_2^-, and NO_3^-
Ke *et al*., 1959	*o*-Nitrobenzoic acid	Growth: 0.1%; Oxygen uptake: 2 mM	*Flavobacterium* from soil	Growth: 18-20 hours; oxygen uptake: 2.5 hours	Growth, oxygen uptake
Smith *et al*., 1968	*p*-Nitrobenzoic acid; 2-fluoro-, 2-chloro-, 2-bromo-4-nitrobenzoate	10. gm/ℓ	*Nocardia erythropolis* grown on *p*-nitrobenzoate	Growth: 4 days; Oxygen uptake: 2 hours	Growth, oxygen uptake
Symons *et al*., 1961	*o*-, *m*-, and *p*-Nitro-Na benzoate, 3,5-dinitro-Na benzoate, 2,4,6-trinitro-Na benzoate	C.O.D. of 125 mg/ℓ	Nitrobenzoate-adapted activated sludge	6 Hours, oxygen uptake; 24 hours, nitrogen release, up to 54 days in case of di- and tri-nitro substituted compounds	Oxygen uptake, nitrogen release, C.O.D. reduction
Tsukamura, 1954 (Abstract)	*p*-Nitrobenzoic acid	0.015%	*Mycobacterium tuberculosis*	--	Formation of arylamine
Villanueva, 1960	*o*-, *m*-, *p*-Nitrobenzoic acid	0.05% (w/v)	*Nocardia* V	16 Days	Growth

for o- and p-nitrobenzoic acid, 4.23 and 5.62 respectively, Cain, 1958). Based on these findings, the author proposed that the fate of nitrobenzoic acids in the natural environment will be dependent on the pH value of the environment.

The ease with which an organism can be enriched on a chemical from a natural population of microorganisms can sometimes provide a fairly good indication of the relative biodegradability of the chemical in the environment. For example, Cartwright and Cain (1959 a) experienced a considerable amount of difficulty in isolating organisms capable of metabolizing the meta-isomer of nitrobenzoic acid, although microorganisms utilizing o- and p-nitrobenzoate were isolated with ease. This may be interpreted to mean that the o- and p-isomer would disappear from the environment far more easily than the meta-isomer. This conclusion agrees with the results of a number of studies with pure and mixed cultures and with natural populations. For example, in a study where soil served as the source of microorganisms, the meta-isomer persisted for over 64 days whereas o- and p-isomers were found to be degraded rapidly (Alexander and Lustigman, 1966). The relative rates of o-, m-, and p-nitrobenzoic acid degradation by activated sludge were determined by Symons et al. (1961); the o- and p-position compounds were metabolized almost at the same rate, with the meta-position compound again being the slowest (Table 36). Of the 34 strains of soil bacteria belonging to the Pseudomonas group tested for their ability to metabolize various isomers of nitrobenzoic acid, some were capable of utilizing p-nitrobenzoic acid, but none of the 34 was active on o- or m-nitrobenzoate (Kameda et al., 1957) (Table 37). Villanueva (1960) studied the ability of nitroaromatic compounds

Table 36. Time to Reach 100 mg/ℓ Level of Soluble C.O.D. Based on Projected Curves (Symons _et al_., 1961)

Compound	Time Required for Degradation (Days)
_o_-Nitro Na benzoate	5.2
_p_-Nitro Na benzoate	5.5
_m_-Nitro Na benzoate	46.0

* Initial COD concentrations were in the range of 630 - 690 mg/ℓ.

Table 37. Metabolic Activities of 34 Strains of Soil Bacteria Belonging to _Pseudomonas_ Group Towards Derivatives of Benzoic Acid (Kameda _et al_., 1957)

Benzoic Acid Derivative Tested	Number of Strains Which Showed Visible Growth	Number of Strains Which Failed To Grow	Strains Not Tested
_p_-Nitrobenzoic acid	3	31	0
_m_-Nitrobenzoic acid	0	33	1
_o_-Nitrobenzoic acid	0	33	1

to act as sole source of carbon and nitrogen for a species of Nocardia (referred to as Nocardia V) and found that only p-nitrobenzoic acid promoted full growth; the o- and m-isomer gave much smaller growth yield in the same period.

The results of the laboratory biodegradation experiments indicate that some removal of o- and p-isomer of mono nitrobenzoic acids would probably occur in the environment. The m-isomer has consistently been shown to be persistent in pure culture, mixed culture, and enrichment culture tests, and in view of this information it appears unlikely that the m-isomer would degrade to a measurable extent in the natural environment.

Routes of Degradation

Nitrobenzoic acid has been reported to degrade by different pathways depending upon microorganisms. The major route of breakdown of nitrobenzoic acids in Nocardia sp. was suggested to be via oxidation by way of hydroxylated intermediates. From the studies on the effect of known metabolic inhibitors on the oxidation of nitrobenzoic acid and through application of simultaneous adaptation methods, Cartwright and Cain (1959 a and b) have suggested the scheme for oxidative metabolism of nitrobenzoates depicted in Figure 16.

Although aryl amines are formed in the culture fluid under certain conditions, they have been shown not to lie on the direct oxidation pathway of nitrobenzoic acid in Nocardia sp. This is also supported from the experiments of Villanueva (1960), who found that Nocardia V., which grows on p-nitrobenzoic acid, fails to grow when provided with p-amino

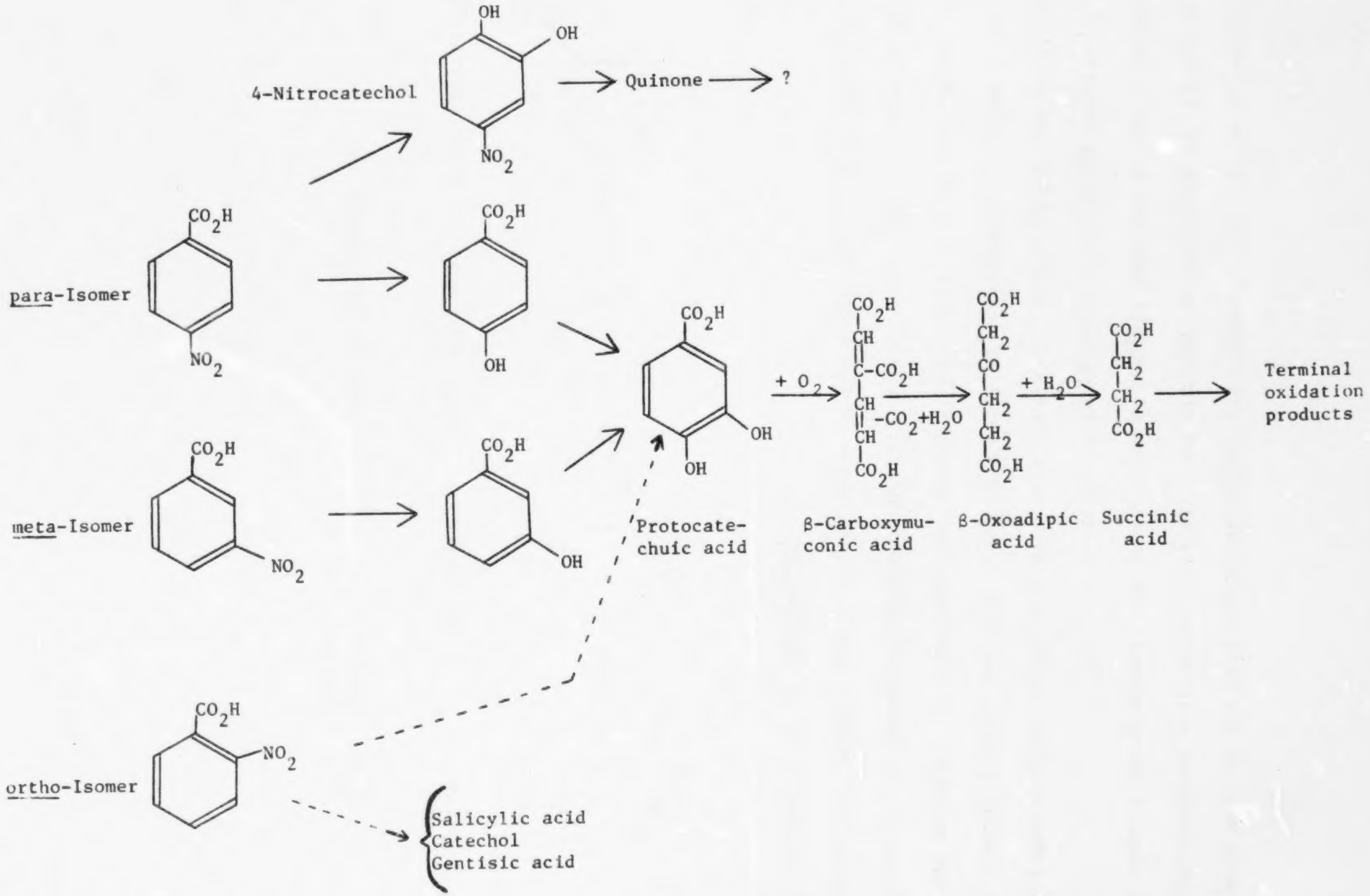


Figure 16. Metabolic Pathways of Degradation of Nitrobenzoate by Species of the Genus Nocardia (Cartwright and Cain, 1959 a)

benzoic acid as the sole source of carbon and nitrogen. During the metabolism of nitrobenzoic acid by *Nocardia* sp., the nitrogen is liberated as nitrite in the case of the *m*-isomer, and in the form of ammonia with the *o*- and *p*-isomer.

A strain of *Pseudomonas fluorescens* capable of utilizing *p*-nitrobenzoic acid as a sole source of organic carbon and nitrogen for aerobic growth, has been found to metabolize nitrobenzoic acid via a different pathway. In this organism, *p*-aminobenzoic acid is a direct intermediate in the breakdown of *p*-nitrobenzoic acid (Durham, 1958). Based on the experimental result, the following sequence was suggested for *p*-nitrobenzoate transformation by *P. fluorescens*.

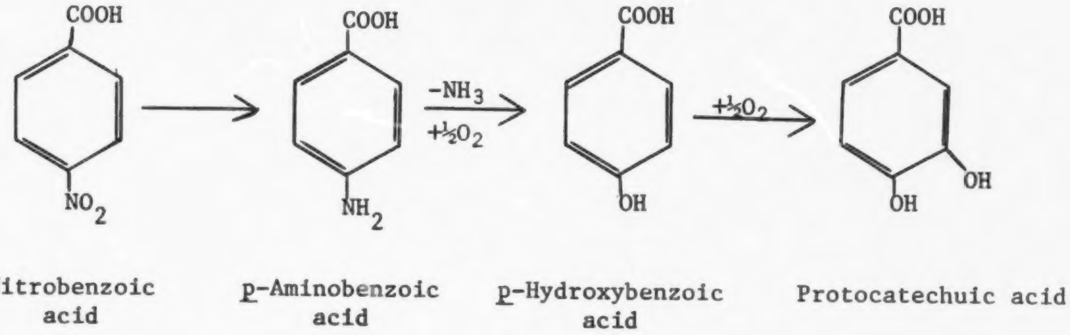


Figure 17. Metabolism of *p*-Nitrobenzoate by *Pseudomonas* sp. (Durham, 1958)

Further metabolism of protocatechuic acid was suggested to be via β-oxoadipic acid as reported by Stanier et al. (1950) and shown in Figure 16. Symons et al. (1961) have reported a similar pathway of p-nitrobenzoate degradation by activated sludge.

The o-isomer of nitrobenzoic acid, on the other hand, has been shown to be metabolized in Flavobacterium sp. via intermediates nitrosobenzoic acid and hydroxyl amino benzoic acid without the involvement of o-aminobenzoic acid (Ke et al., 1959).

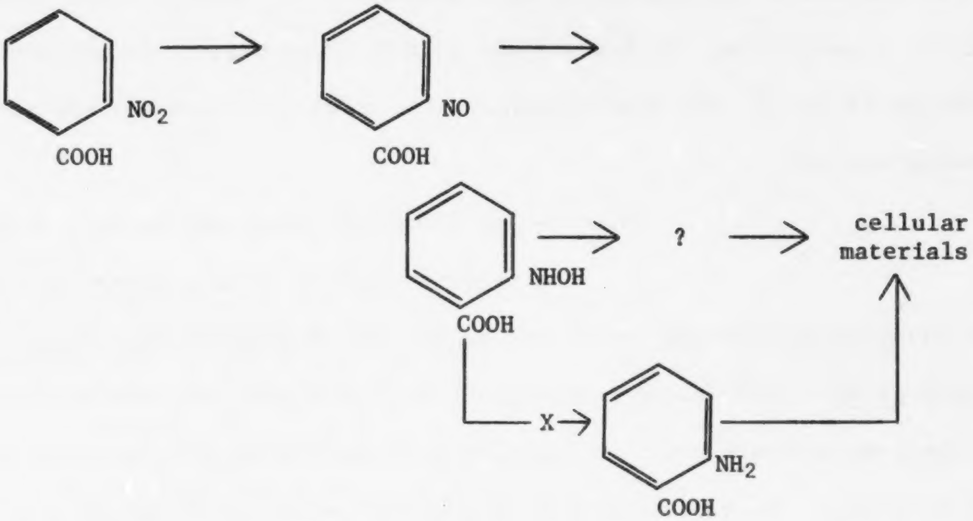


Figure 18. Metabolism of o-Nitrobenzoate by Pseudomonas sp. (Ke et al., 1959)

o-Nitrobenzoic acid metabolism by activated sludge was also shown to proceed via a similar pathway (Symons et al., 1961). Symons and his co-workers further reported that the intermediates which followed included o-hydroxylaminobenzoic acid and possibly catechol, protocatechoic acid and succinic acid. The results of Ke et al. (1959), however, suggest that protocatechuic acid does not occupy an intermediary position in the metabolism of the o-isomer.

As indicated above, the routes of degradation of nitrobenzoic acid by pure cultures of microorganisms appear to have been fairly well investigated. However, no studies have been reported in the literature which deal with the route of breakdown under environmental conditions. It is uncertain if the degradation of nitrobenzoic acid in the environment will be catalyzed predominantly by Nocardia sp., Pseudomonas sp. or by some other species of microorganisms which has not yet been enriched, or by a mixture of these microorganisms. Furthermore, it is also uncertain if the pathway of degradation by an organism will be similar under laboratory and field conditions. In the absence of this information, the route of degradation of o-, m-, and p-nitrobenzoic acid under environmental conditions remains obscure.

(b) Di- and Trinitro-substituted Benzoic Acids

The studies reported to date reveal that di- and trinitro-substituted benzoic acids are not attacked by microorganisms. Symons et al. (1961) noted that neither 3,5-dinitro-Na-benzoate nor 2,4,6-trinitro-Na-benzoate could be degraded by activated sludge adapted to p-nitrobenzoate. Degradation could also not be initiated by introducing an easily metabolizable carbon source (e.g., mononitrobenzoic acids or by addition of fresh sludge to supply new microorganisms and nutrients). The studies undertaken by Chambers et al. (1963) further support the inability of microorganisms to degrade di- and trinitro-substituted benzoic acids. These authors tested the ability of a culture highly adapted to degrade phenol (phenol was presumed to have the same basic configuration as nitrobenzoates, and thus be able to induce requisite enzymes) to attack various nitrobenzoic acids. The experimental findings indicated little or no activity of the phenol-adapted cells with 3,5-, 3,4-, or 2,5-dinitrobenzoic acid, or with 2,4,6-trinitrobenzoic acid, which suggests that these compounds are difficult to degrade. It must be pointed out, however, that nonbiodegradability of nitrobenzoates in this study may be attributed to the inability of phenol to induce appropriate enzymes for the degradation of di- and trinitro-substituted benzoic acids. Cain (1958), on the other hand, has noted that one of the dinitro-substituted compounds - 2,4-dinitrobenzoic acid - could serve as the sole carbon source to the species of *Nocardia* enriched and grown on *p*- or *o*-nitrobenzoates. However, since only a small amount of growth was observed after the test period of seven days, the biodegradable nature of the dinitro-substituted benzoic acid is somewhat inconclusive.

(c) Halogen Analogues of Nitrobenzoic Acids

A number of studies dealing with biodegradation of halogen analogues of nitrobenzoic acids have been reported. The utilization of halogen-substituted nitrobenzoates by *p*-nitrobenzoate-grown cells of *Nocardia erythropolis* was examined by Smith *et al*. (1968). The authors found that 2-fluoro-, 2-chloro-, or 2-bromo-4-nitrobenzoate did not support growth of this organism, nor did they increase the growth yield when added to the cultures utilizing fumarate as carbon source. The halogenated nitrobenzoic acid, when present together with *p*-nitrobenzoic acid, inhibited the induction of the *p*-nitrobenzoate-oxidation system, and caused inhibition of growth. The halogen analogues of *p*-nitrobenzoates were, however, oxidized by washed *p*-nitrobenzoate-grown cells of *N*. *erythropolis* (Cain *et al*., 1968). The ease of oxidation decreased in the order *p*-nitrobenzoate>

2-fluoro-4-nitrobenzoate>2-chloroderivative>2-bromo and 2-iodo derivatives (Figure 19).

For detailed metabolic study, Cain and his co-workers used only one of the halogen analogues: 2-fluoro-4-nitrobenzoate. The results indicated that 2-fluoro-4-nitrobenzoate was oxidized to fluoroacetate; the pathway of degradation appeared similar to that reported for p-nitrobenzoate (Cartwright and Cain, 1959 a, see Figure 16), except that corresponding fluoro-intermediates were formed. Several fluorine containing metabolic intermediates were detected by the authors, and 2-fluoroprotocatechuate was identified as one of them. No fluoride ions were released into the incubation medium. The nitrogen of the nitro group in 2-fluoro-4-nitrobenzoate was recovered entirely as ammonia. Incubation of p-nitrobenzoate grown cells with 3-fluoro-4-nitrobenzoate and 3-methyl-4-nitrobenzoate resulted in the formation of o-dihydroxy compounds: 5-fluoroprotocatechuate and 5-methylprotocatechuate, respectively. Further breakdown of these compounds was not reported.

(iii) Nitrophenols and Related Compounds

Nitrophenols are generally toxic and have a pronounced inhibitory effect upon the processes of assimilation in cell metabolism. For example, the selective action of dinitrophenol on cell respiration is well known (Simon and Blackman, 1953). These properties of nitrophenols, coupled with the fact that a number of nitrophenols are commercialized as pest control agents or are used for synthesis of other more selective pesticidal agents, have inspired both the basic scientist as well as those concerned with the pollution potential of nitrophenols from their commercial applications, to investigate the biological transformation of these compounds. The fate of

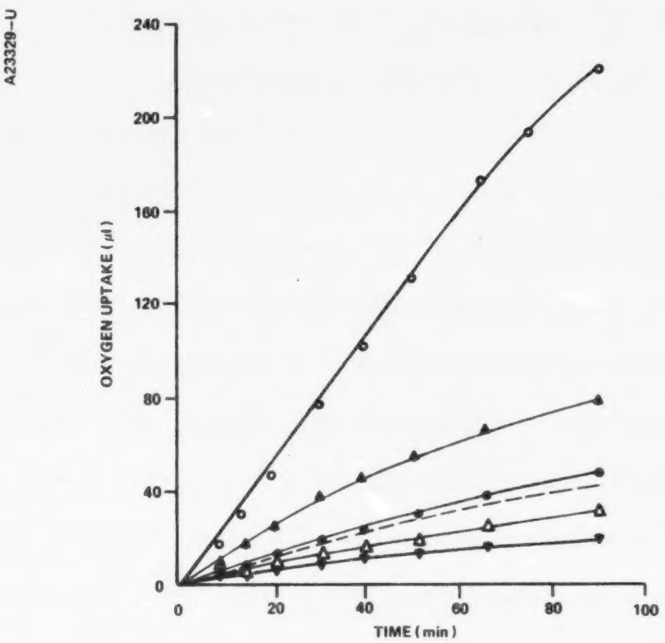


Fig. 19. Oxidation of halogenonitrobenzoates by p-nitrobenzoate-grown *N. erythropolis*. o, p-Nitrobenzoate; ▲, 2-fluoro-4-nitrobenzoate; ●, 2-chloro-4-nitrobenzoate; Δ, 2-bromo-4-nitrobenzoate; ▼, 2-iodo-4-nitrobenzoate; ----, 3-fluoro-4-nitrobenzoate. Oxygen uptakes were corrected for endogenous respiration (185 µl/hr.). (Cain *et al.*, 1968)

nitrophenols and the routes of their degradation have thus been reasonably well investigated. The experimental details of the studies dealing with the decomposition of nitrophenols with pure cultures or with natural communities of microorganisms are summarized in Table 38.

(a) o-, m-, and p-Nitrophenols

Microbial Degradation

As can be noted from the table, in studying the environmental fate of nitrophenols, efforts of the majority of researchers have been directed towards isolation of the microorganism(s) which can modify these chemicals either by using them as sources of energy, carbon, or nitrogen, or by cometabolism (concomitant metabolism of a non-growth substrate). Simpson and Evans (1953) isolated two organisms (Pseudomonas sp.), which used o-nitrophenol (referred to as strain SO) and p-nitrophenol (referred to as strain SP) as the sole source of carbon. The organisms were isolated from the filter beds of a biological treatment plant. The nitro group was oxidatively eliminated as nitrite in this process. An organism which appeared to be a strain of either Flavobacterium or a closely related bacterium, capable of using p-nitrophenol as carbon and energy source, has been isolated from soil by Raymond and Alexander (1971). The organism required some component of soil extract for its multiplication in the nitrophenol medium. In addition, the medium employed for studying degradation of nitrophenol also contained small quantities of yeast extract. Cells grown on p-nitrophenol were able to cometabolize the m-isomer to nitrohydroquinone but were not able to use it as a carbon source for growth.

In a number of pure culture studies dealing with the degradation of nitrophenols, researchers have used organisms which

Table 38. Experimental Conditions Used by Various Investigators in Studying the Fate of Nitrophenols and Related Compounds

Nitrophenols

Reference	Chemicals Tested	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Alexander and Lustigman, 1966	*o*-, *m*-, *p*-Nitrophenol	8-15 μg/ml	Niagara silt loam	64 Days	Loss of UV absorption
Cain, 1958	*o*-, *m*-, *p*-Nitrophenols	0.025%	*Nocardia sp*. enriched on *p*-nitrobenzoic acid	7 Days	Growth
Chambers *et al*., 1963	*o*-, *m*-, *p*-Nitrophenol; 2,4-, 2,6-dinitrophenol; 2,4,6-,trinitrophenol; 2-chloro-4-nitrophenol; 4-chloro-2-nitrophenol; 2,6-dichloro-4-nitrophenol	60-100 mg/ℓ	Microorganisms from soil, compost, or mud from a catalytic cracking plant waste lagoon, adapted to phenol	180-230 min.	Oxygen uptake
Guillaume *et al*., 1963	*m*- and *p*-Nitrophenol	50 μg/ml	*Mycobacteria* sp.	--	Formation of the product-4-nitropyrocatechol
Gundersen and Jensen, 1956	*o*-, *m*-, *p*-Nitrophenol; 2,4-, 2,5-, and 2,6-dinitrophenol; 2,4,6-trinitrophenol	0.01-0.05%	*Arthrobacter simplex* isolated from soil	Up to 10 days	Growth, nitrite release, loss of color
Jensen and Lautrup-Larsen, 1967	2,4-Dinitrophenol; 2,4,6-trinitrophenol; *o*-, *m*-, *p*-nitrophenol; 2-methyl-4-nitrophenol; dinitro(*sec*)butylphenol	0.2-0.5 mM	*Arthrobacter* and *Pseudomonas*-like organisms isolated from soil	20 Days for growth; 24 hours for nitrite	Growth, formation of nitrite, loss of color
Madhosingh, 1961	2,4-Dinitrophenol	0.001-0.006%	Wood decaying fungus tolerant to DNP: *F*. *oxysporum* and *C*. *micaceus*	30 Days	Reduction of the nitro groups
Raymond and Alexander, 1971	*p*-, *m*-Nitrophenol	5.5×10^{-4} M	*Flavobacterium* sp. isolated from soil	6-72 Hours	Growth, nitrite formation

Table 38. Experimental Conditions Used by Various Investigators in Studying the Fate of Nitrophenols and Related Compounds (Cont'd)

Reference	Chemicals Tested	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Nitrophenols (cont.)					
Simpson and Evans, 1953	*o*-, *p*-Nitrophenol; 2,4-dinitrophenol	100 ppm	*Pseudomonas* sp. isolated from the filter beds of a biological detoxification plant	--	Growth
Villanueva, 1960	*o*-, *m*-, *p*-Nitrophenols; 2,4-dinitrophenols; 2-amino-4-nitrophenol; picric acid	0.025%	*Nocardia* V	16 Days	Growth
Villeret, 1965	*o*-, *m*-, *p*-Nitrophenols	10^{-5}-10^{-2} M	*Chlorella vulgaris*	--	Growth with nitro group as the nitrogen source
Nitrocresols					
Chambers *et al*., 1963	4,6-Dinitro-*o*-cresol; 2,4,6-trinitro-*m*-cresol	60-100 mg/ℓ	Microorganisms from soil, compost or mud from a catalytic cracking plant waste lagoon, adapted to phenol	210 Minutes	Oxygen uptake
Gunderson and Jensen, 1956	4,6-Dinitro-*o*-cresol	0.02%	*Arthrobacter Simplex* isolated from soil	Up to 10 days	Growth, nitrite release, loss of color
Jensen and Lautrup-Larsen, 1967	4,6-Dinitro-*o*-cresol	0.2-0.5 mM	*Arthrobacter* and *Pseudomonas*-like organisms isolated from soil	20 Days for growth; 24 hours for NO_2^- release	Growth, release of nitrite, loss of color
Tewfik and Evans, 1966	4,6-Dinitro-*o*-cresol	0.01% (w/v)	*Pseudomonas* sp. isolated from garden soil	--	Growth, release of nitrite ion

Table 38. Experimental Conditions Used by Various Investigators in Studying the Fate of Nitrophenols and Related Compounds (Cont'd)

Nitroresorcinols

Reference	Chemicals Tested	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Brebion *et al.*, 1967 (Abstract)	2,4-Dinitroresorcinol; 2,4,6-trinitro resorcinol	Approx. 200 ppm	Bacterial cultures taken from earth, water, and mud, especially from areas already polluted	--	Elimination of the parent compound
Chambers *et al.*, 1963	2,4,6-Trinitroresorcinol	60 mg/ℓ	Microorganisms from soil, compost, or mud from a catalytic cracking plant waste lagoon, adapted to phenol	210 Minutes	Oxygen uptake

were enriched on chemical compounds resembling nitrophenols. For example, Cain (1958) tested the ability of the Nocardia sp. isolated by enrichment on p-nitrobenzoic acid to utilize o-, m-, and p-nitrophenols. The authors noted no growth of the organism on any of the nitrophenol isomers. The microorganisms which were enriched from soil with herbicide 4,6-dinitro-o-cresol were capable of decomposing p-nitrophenol with release of nitrite; other nitrophenol isomers were not attacked (Gundersen and Jensen, 1956; Jensen and Lautrup-Larsen, 1967) (Table 39). Chambers *et al*. (1963) noted that a culture adapted to degrade phenol was capable of oxidizing o-, m-, and p-nitrophenol at a slow rate; o- and m-isomers were oxidized at relatively higher rates than the p-isomer. The culture was also able to oxidize a number of chloro-substituted nitrophenols at slow rates. The compounds attacked were 2-chloro-4-nitrophenol, 4-chloro-2-nitrophenol and 2,6-dichloro-4-nitrophenol, the rate of oxidation being the highest with 4-chloro-2-nitrophenol. The

Table 39. Degradation of Mononitrophenols by DNOC-Grown Bacteria (Gundersen and Jensen, 1956; Jensen and Lautrup-Larsen, 1967)

Organism Enriched on Herbicide 4,6-Dinitro-*ortho*-cresol	Nitrophenols Attacked		
	o-Nitrophenol	m-Nitrophenol	p-Nitrophenol
Arthrobacter Simplex	-	-	+
Arthrobacter X	-	-	-
Pseudomonads (from acid loam field soil)	-	-	+

assessment of biodegradation from the oxygen uptake data is susceptible to many criticisms. For example, by their uncoupling action, nitrophenols may stimulate the endogenous oxygen uptake rates which may be confused with increased oxygen uptake due to the breakdown of nitrophenol. One of the well-known uncouplers of oxidative phosphorylation is 2,4-dinitrophenol; however, a similar action may also be exerted by mononitro- and certain trinitrophenols.

Several researchers have investigated the biodegradability of nitrophenols using the microorganisms which were selected on the basis of metabolic versatility. Guillaume *et al*. (1963) studied the oxidation of *p*- and *m*-nitrophenol by certain *Mycobacteria*. The experimental findings revealed that both the isomers could be attacked and that product of oxidation was identified as 4-nitropyrocatechol. Since some species of the genus *Nocardia* were reported to utilize aromatic compounds (*Bergey's Manual of Determinative Bacteriology*, 1948), Villanueva (1960) tested the ability of a *Nocardia* sp. (referred to as *Nocardia* V) to utilize nitrophenols for growth. The results of this study are, however, inconclusive perhaps because the estimation of growth was based on the visual examination of the culture. Villeret (1965) reported the utilization of the nitro group of *m*-, *p*-, and *o*-nitrophenols as a source of nitrogen during growth of freshwater algae *Chlorella vulgaris*. The *m*-isomer supported better growth than the *p*-isomer, with the *o*-isomer giving minimal growth.

The studies described above, in which pure cultures of microorganisms obtained from a variety of sources have been used, appear to suggest that mononitrophenols are susceptible to microbial attack. The *p*- and *o*-isomer are attacked by a greater number of microorganisms than the *m*-isomer. Whether mononitrophenols are degraded in the natural environment, and whether the order of biodegradability is the same as derived from the laboratory pure culture studies, is debatable. The main difficulty encountered in extrapolation of the pure culture data is that the concentration of the test chemical employed for enrichment of an organism and for obtaining a reasonable amount of cell growth at the expense of the chemical is far removed from the concentrations generally encountered in nature. Whether the appropriate enzyme(s) can be induced and/or growth can occur at low environmentally significant concentrations is not clearly understood.

There are only a few published studies regarding the breakdown of nitrophenolic compounds by natural communities of microorganisms. Brebion *et al*. (1967) examined the ability of the microorganisms taken from soil, water, or mud, and grown in a porous mineral bed to attack *p*-nitrophenol. The cells were cultivated on a mineral nutrient solution in which nitrophenols were added as the sole source of carbon. The experimental findings revealed no significant removal of the compound under these conditions. The fate of *p*-, *m*-, and *o*-nitrophenols by natural communities of microorganisms in soil has been studied by Alexander and Lustigman (1966). These authors found that the *o*-isomer was far more resistant to degradation (persisted for >64 days) than the *m*- and *p*-isomer (*m*, 4 days; *p*, 16 days). This is unlike the observations made with pure cultures of microorganisms where the *o*-isomer was found to be attacked easily. The degradation of the test compounds in this study was determined by following the loss of ultraviolet absorbancy when the benzene ring is cleaved by microorganisms present in the soil. The assay technique makes use of a small soil inoculum to minimize interference, and thus, in turn, limits the concentration of microorganisms and soil nutrients in the assay system. Furthermore, the test conditions used in this study simulate a soil suspension rather than the soil. In view of these shortcomings, extrapolation of the laboratory data to assess environmental fate of nitrophenols is difficult.

Routes of Degradation

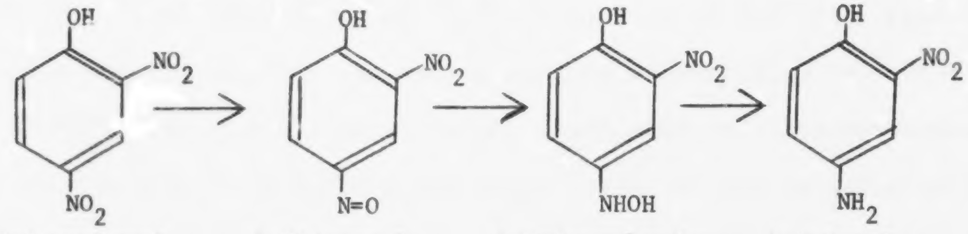
Very little is known about the metabolic sequence of the breakdown of o-, m-, or p-nitrophenol. The available information has been derived predominantly from the pure culture work. Simpson and Evans (1953) using *Pseudomonas* sp. which utilize o-nitrophenol as its sole source of organic carbon, obtained evidence which supported the view that degradation of o-nitrophenol proceeded via an oxidative elimination of the nitro group and resulted in the formation of catechol. The catechol thus formed was suggested to be utilized by well-known pathways involving *cis*-muconic and β-ketoadipic acid (Evans *et al.*, 1951). Similarly, in the metabolism of p-nitrophenol, the corresponding dihydroxy intermediate formed was shown to be p-dihydroxybenzene (quinol). Raymond and Alexander (1971) reported a somewhat different pathway for nitrophenol metabolism in a *Flavobacterium* sp. These authors obtained evidence which suggested that an initial step in the reaction with nitrophenol is its hydroxylation. The metabolite generated from p-nitrophenol was identified as 4-nitrocatechol; the metabolite underwent further degradation as evidenced by the considerable amount of oxygen consumption and growth of the bacterium on p-nitrophenol. The actual metabolites were, however, not directly identified in this study. The product of cometabolism of the m-isomer was shown to be nitrohydroquinone; the metabolite was not degraded further by the Flavobacterium sp. A similar mechanism of oxidation for nitrophenol has been reported in certain Mycobacteria (Guillaume et al., 1963).

(b) Di- and Trinitrophenols

The most extensively investigated compounds in this group are 2,4-dinitrophenol (2,4-DNP) and 2,4,6-trinitrophenol (picric acid). As early as 1953, Simpson and Evans (1953), in a brief communication, reported isolation of an organism from soil which formed nitrite from 2,4-dinitrophenol. A number of microorganisms enriched from soil on dinitro-o-cresol were shown to metabolize the nitro group of 2,4-dinitrophenol and 2,4,6-trinitrophenol with the formation of nitrite; 2,5- and 2,6-dinitro-substituted phenols were not attacked and only negligible amounts of nitrite appeared with these compounds (Jensen and Lautrup-Larsen, 1967; Gundersen and Jensen, 1956). The picture is somewhat obscure as far as the carbon nutrition from nitrophenolic compounds is concerned. It is unclear from the results if the microorganisms cause ring cleavage and/or derive any carbon from the compound. One of the organisms in this study was identified as Arthrobacter simplex; other organisms were provisionally referred to as Arthrobacter x and Pseudomonas. The percentages of organic nitrogen converted to nitrite-nitrogen from different compounds were different. Whereas nearly all the trinitrophenol - nitrogen was converted to nitrite, only 50% could be detected from 2,4-dinitrophenol. The fate of the remaining nitrogen in the case of 2,4-dinitrophenol is obscure, except that a small portion was found to be assimilated by the organism (Jensen and Lautrup-Larsen, 1967). The metabolic attack on 2,4,6-trinitrophenol was accompanied by formation of an unidentified soluble rust brown pigment (structure unknown). A similar pigment was reported to be formed in the cultures of *Arthrobacter simplex* incubated with 2,5-dinitro- and 2,6-dinitrophenol.

The experimental findings of Chambers *et al.* (1963) indicate that a phenol-adapted culture was able to slowly oxidize 2,4- and 2,6-dinitrophenol and 2,4,6-trinitrophenol. The oxidative activity was slowest with the 2,6-dinitro isomer. The results of oxygen uptake studies with nitrophenolic compounds must be interpreted with caution as discussed earlier. The possibility cannot be excluded that the increased oxygen uptake observed by the authors was due to the enhancement of endogenous oxygen uptake by nitrophenols due to their uncoupling effect.

A number of studies have indicated that enzyme systems in bacteria and fungi can reduce dinitrophenolic compounds to the corresponding arylamines in an attempt to detoxify the compounds. For instance, the aerobic bacterium *Azotobacter chroococcum*, anaerobic bacterium *Clostridium butyricum*, and the fungus *Fusarium* (a DNP-tolerant fungus) may all reduce 2,4-DNP according to Radler (1955), Lehmber (1956), and Madhosingh (1961). The reduction of the nitro group in the fungus was postulated to take place in stages, involving the intermediate formation of the nitroso and hydroxylamino groups as shown in Figure 20.



Dinitrophenol

4-Nitroso-2-nitrophenol

4-Hydroxylamino-2-nitrophenol

4-Amino-2-nitrophenol

Figure 20. Sequence of the Reduction of Nitro Group in *Fusarium* sp. (Madhosingh, 1961)

The reduction of the nitro group could occur either in position 2 or 4, thus giving rise to 2-amino-4-nitrophenol and 4-amino-2-nitrophenol, respectively. Both of these products have been identified by Madhosingh (1961) in the DNP-treated media which had maintained the growth of the fungus. No other information regarding the pathway of degradation or detoxification of dinitro- and trinitrophenolic compounds is available in the literature.

From the experimental evidence presented above, it can be concluded that the nitro compounds 2,4-dinitrophenol and 2,4,6-trinitrophenol are susceptible to partial degradation by certain microorganisms. It may be speculated that nitrophenols will be subjected to microbial attack in the environment by the microorganisms adapted to phenol or the herbicide dinitro-_o_-cresol. Both phenol and dinitro-_o_-cresol appear to be widespread contaminants and thus the microorganisms adapted to them will also be expected to be widespread. Since 2,5- and 2,6-dinitro-substituted phenols have not been shown to be altered by DNOC- or phenol-adapted culture, it appears likely that these isomers may persist in the environment for extended periods of time.

(c) Nitrocresols

A number of nitrocresols are well-known herbicides, and their fate in soil has received much attention. DNOC (4,6-dinitro-_o_-cresol) usually disappears from the soil within a few weeks to two months (Petersen and Hammarlund, 1953; Bruinsma, 1960; Jensen, 1966). The elimination of DNOC at least in part was attributed to the effect of microorganisms (Jensen and Lautrup-Larsen, 1967). Jensen and coworkers (Gundersen and Jensen, 1956; Jensen and Lautrup-Larsen, 1967) isolated an _Arthrobacter_ and a Pseudomonas that grew on DNOC with the release of nitrite. The Pseudomonas sp. was able to attack DNOC within a wide range of pH (4.4 - 8.8). The authors reported that certain strains of Nocardia isolated from 2,6-dinitrophenol-treated soil could also metabolize DNOC. A Pseudomonas capable of metabolizing DNOC in the presence of hydrogen donors (e.g., glutamate, lactate, yeast extract, etc.) has been isolated from soil by Tewfik and Evans (1966). To some extent dinitro- and trinitro-substituted cresols were oxidized by a culture which had been adapted to degrade phenol (Chambers *et al.*, 1963). The compounds investigated in this study were 4,6-dinitro-*o*-cresol and 2,4,6-trinitro-*m*-cresol. The oxidative activity was found to be relatively greater with dinitro- than with trinitrocresol.

The degradation pathway of dinitro-*o*-cresol in pure cultures of microorganisms has been investigated by Tewfik and Evans (1966). The findings indicated that in Pseudomonas sp., degradation proceeded via formation of an aminocresol; in contrast, Arthrobacter simplex was found to employ initially a reaction involving a hydroxylated intermediate (Figure 21). The evidence indicated that the pathway of degradation after the inital step was similar in both organisms.

(d) Nitroresorcinol

Chambers *et al.* (1963) investigated the susceptibility of 2,4,6-trinitroresorcinol to microbial attack using a culture which had been adapted to degrade phenol. The criterion for degradation was the oxidation of the molecule by microbial action. The experimental findings indicated that negligible amounts of oxygen were consumed with trinitroresorcinol as the substrate, suggesting the recalcitrant nature of the molecule. It

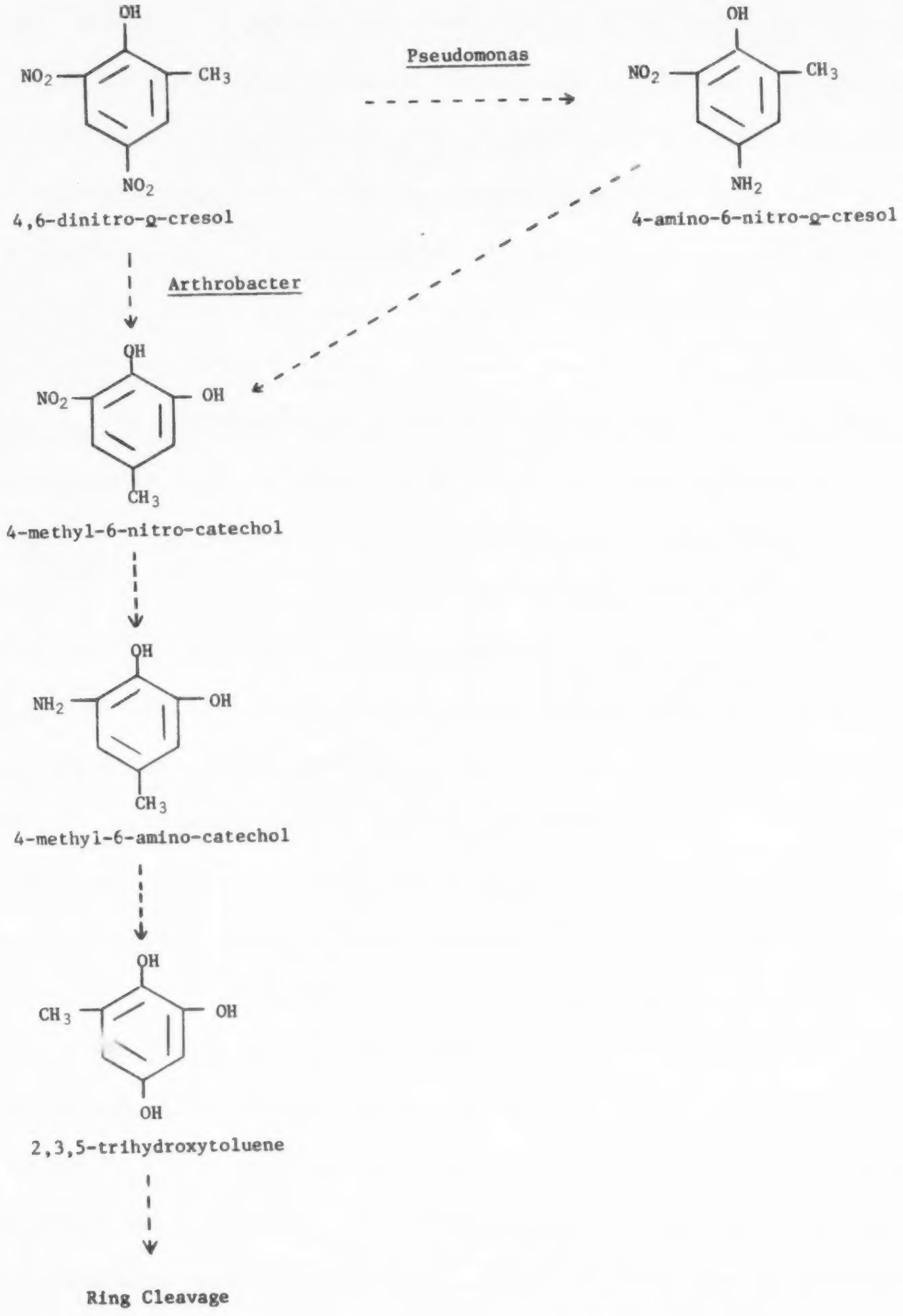


Figure 21. Metabolism of 4,6-Dinitro-o-Cresol by Soil Microorganisms (Tewfik and Evans, 1966)

is also likely, however, that adaptation of the microorganisms to phenol failed to induce appropriate enzymes for the degradation of trinitroresorcinol. The inability of microorganisms to attack 2,4,6-trinitroresorcinol was also indicated from the studies of Brebion et al. (1967). These investigators incubated the microorganisms (from soil, water, or mud especially from areas already polluted) with a nutrient solution to which nitro-substituted resorcinols were added as the sole carbon source. The incubation was carried out in a porous mineral bed. The experimental findings revealed no significant removal of 2,4,6-trinitroresorcinol and about 36% removal of 2,4-dinitroresorcinol. The results of the studies of both Chambers et al. (1963) and Brebion et al. (1967) point to the recalcitrant nature of the 2,4,6-nitro-substituted resorcinol. On the other hand, the 2,4-nitro-substituted compound may be degraded to a small extent.

(iv) Nitrotoluenes

There has been a considerable amount of concern over the level of trinitrotoluene pollution generated by military munitions production. In order to minimize water pollution, a number of researchers have investigated the biodegradability of trinitrotoluene and other explosives and the feasibility of using biological processes for treatment of the waste water resulting from their manufacturing and loading in munitions products. The studies can be classified into three major categories, depending upon the choice and source of biological agent and environmental conditions used in degradation:

(a) Degradation by pure cultures of microorganisms.

(b) Degradation by natural communities of microorganisms.

(c) Degradation under sewage treatment plant conditions.

All the studies dealing with the environmental fate of nitrotoluenes are summarized in Table 40.

(a) Degradation by Pure Cultures of Microorganisms

As early as 1951, Russian worker Rogovskaya noted that certain microorganisms can utilize TNT better as a source of nitrogen than as a source of carbon. Enzinger (1970) isolated several Pseudomonas-like microorganisms from the mixed liquor suspended solids of a TNT laboratory scale biooxidation unit and from contaminations found in prepared TNT agar; the organisms could be acclimated to TNT and grown successfully on trypticase soy broth in the presence of high concentrations of TNT (29-100 ppm) (one of the organisms grew without prior acclimation). Acclimation was achieved by subculturing into media containing successively higher TNT concentration. TNT concentration during growth could be reduced to as low as 1.25 ppm (from the starting concentration of 100 ppm) within a 5 day period; the reduction in the concentration of TNT was linked to the appearance of two unidentified products in the medium. No unaltered TNT was detected in the cells. In addition to testing the pure cultures of microorganisms isolated as described above, the author also investigated the ability of Zoogloea ramigera 115 to degrade TNT. The organism is relatively abundant in sewage treatment facilities and has been isolated by Friedman and Dugan (1968). The cells of Zoogloea were found to be relatively more sensitive than Pseudomonas to TNT. It is unclear from the paper, however, if Zoogloea was able to remove TNT after an acclimation period.

Three Pseudomonas-like organisms capable of metabolizing TNT have been isolated by Won et al. (1974) from mud and water

Table 40. Salient Features of the Biodegradation Studies with Nitrotoluenes

Reference	Test Compound	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Villanueva, 1960	*p*-Nitrotoluene	0.025% (w/v)	*Nocardia* V	16 Days	Growth
Chambers *et al.*, 1963	2,4-Dinitrotoluene; 2,4,6-trinitrotoluene	100 mg/ℓ	Microorganisms in soil, compost, or mud from a catalytic cracking plant waste lagoon, adapted to phenol	3 Hours	Oxygen uptake
Osmon and Klausmeier, 1973	Trinitrotoluene, ammonium picrate	100 mg/ℓ	Sewage effluent, TNT loading facility effluent, soil, pond, and aquarium water, pure culture of *Pseudomonas aeruginosa*	4-6 Days	Assay of the parent compound
Won *et al.*, 1974	2,4,6-Trinitrotoluene	12-35 x 10^{-5} M	Mud and water sample obtained from U.S. Naval Ammunition Depot at MacAlester, Okla.	Oxygen uptake: 4 hours; growth: 70 hours	Growth, oxygen consumption
Enzinger, 1970	2,4,6-Trinitrotoluene	100 ppm	Mixed liquor suspended solids from laboratory scale biooxidation units; pure cultures of *Zooglea ramigera* 115	120 Hours	Measurement of the parent compound
Nay, 1972; Nay *et al.*, 1974	Trinitrotoluene manufacturing waste water; 2,4,6-TNT	2.5-40% TNT waste containing 2 mg/ℓ TNT added to the samples; 2,4,6-TNT 12-64 mg/ℓ	Munitions plant domestic waste	8-18 Days	BOD test using manometric apparatus; TNT analysis

samples collected from a naval base. At the naval base, TNT had been used in large amounts for long periods of time, thus enhancing the possibility of selection of TNT degrading microflora. The conversion of TNT was found to vary with the isolates. An isolate (tentatively named as isolate Y) was able to metabolize TNT most effectively. At 24 hours, the TNT concentration was reduced from 100 μg/mℓ to less than 1 μg/mℓ in yeast extract enriched medium; the rates were relatively slower in glucose supplemented cultures. Thin layer chromatographic analysis of the incubation mixture revealed that TNT was metabolized to yield a variety of reduced TNT metabolites and azoxy toluenes (see Figure 22, p. 177); extensive degradation of TNT did not take place since no ring breakdown products were identified. During the enrichment of the microorganisms capable of using TNT, Osmon and Klausmeier (1973) noted the predominance of pseudomonas among the isolated TNT transforming cultures. Consequently, they examined the ability of a pure culture of Pseudomonas aeruginosa (ATCC 13388) to degrade TNT. The organism was found to be able to transform TNT only when provided with extraneous nutrients. For instance, in the presence of 1-2% glucose, TNT concentration was decreased from 100 ppm to 30 ppm in 5 days based on the residual TNT analysis. It was concluded from these observations that degradation occurred via a co-metabolic process (concomitant metabolism of a non-growth substrate). The authors noted the accumulation of small quantities of a number of unidentified TNT metabolites in the medium. The metabolites were reported to be transient in nature and they eventually disappeared. The disappearance of TNT as well of certain TNT metabolites to some extent may be due to their accumulation in the cell. Since the authors did not extract the cells, the extent of removal by this mechanism is unclear.

The pure culture studies described here provide evidence that TNT can undergo minor modification by the action of certain microorganisms; however, any degradation of the compounds is questionable. Furthermore, the concentration of TNT employed in these studies is more representative of the concentration of TNT in the waste water resulting from the TNT-manufacturing process, and thus the results are more applicable to determining the feasibility of biological treatment for disposing of TNT, rather than for assessing its environmental fate.

Very little information is available concerning microbial decomposition of mono- and dinitro-substituted toluenes. Douros and Reid (1956) briefly examined the biodegradability of 2,4-dinitrotoluene using the cultures of soil microorganisms _P_. _aeruginosa_ and _P_. _putida_ which had been adapted to degrade the herbicide 3-(_p_-chlorophenyl)-1,1-dimethylurea. Both strains yielded fair amounts of growth with 2,4-dinitrotoluene as the sole organic compound, which suggests that the compound was attacked by the microorganisms. Villanueva (1960) reported failure of a strain of _Nocardia_ (_Nocardia_ V) to use _p_-nitrotoluene as the sole source of carbon for growth. _Nocardia_ V was chosen for these studies on the basis of the earlier reports regarding catabolic versatility of the genus _Nocardia_ (Moore, 1949; Cain, 1958).

(b) Degradation by Natural Communities of Microorganisms

Osmon and Klausmeier (1973), while attempting to develop a biological disposal method for waste from an explosive factory, studied the ability of inocula from a variety of natural sources to degrade TNT. The following sources of inoculum were tested: effluent from sewage treatment plants; waste water from an ordnance loading facility using TNT; a soil suspension and pond water; and water from a laboratory aquarium. Transformation was studied in a mineral salts medium containing 100 mg/ℓ TNT both alone and in the presence of 1% yeast extract. The microflora from all the sources tested catalyzed a complete and rapid removal of TNT (based on residual TNT analysis) from the yeast extract-enriched medium but not from the medium containing mineral salts and TNT only. The majority of the organisms which showed positive degradative ability on TNT were noted to be *Pseudomonas*-like. The authors also tested the ability of raw sewage and sewage sludge digester supernatant to degrade TNT. The results showed that while raw sewage is ineffective, sludge liquor caused a 64% reduction in the concentration of TNT. Since only the disappearance of TNT was followed in all the above studies, it is not clear if TNT underwent minor modification or was extensively degraded, or to what extent the removal was due to adsorption on the particulate matter.

Chambers *et al*. (1963), in their biodegradation studies with nitro-substituted toluenes, used a culture consisting of several species (*Pseudomonas* predominating) which had been adapted to degrade phenol. Oxygen consumption at the expense of the test substrate was measured using respirometric technique. The ratio of the test oxygen uptake rate to the endogenous rate was found to be nearly 2.5 - 2.6 for 2,4-dinitrotoluene and 2,4,6-trinitrotoluene, implying that these compounds were biodegradable to some degree.

(c) Degradation Under Sewage Treatment Plant Conditions

A considerable amount of work has been reported concerning biological treatability of trinitrotoluene, and of the waste waters resulting from TNT manufacturing operations and from munitions loading plants. Consideration of the fate of these chemicals under waste water treatment plant systems is important because their fate in these systems can be a determining factor in whether the chemicals become environmental pollutants or degrade to innocuous materials.

The early investigations designed primarily to determine the effect of TNT waste on domestic sewage treatment (since non-acclimated samples of activated sludge were used) led researchers (Ruchhoft et al., 1943, 1945 a, b) to conclude that TNT waste should not be treated by either activated sludge or trickling filter units because inhibition of the BOD removal efficiency was observed. The results of the BOD test on TNT-waste revealed that the waste was not biodegradable (upon incubation for up to 129 days, the BOD value was only 11 mg/ℓ as compared to the COD value of 673 mg/ℓ).

Soviet scientists under the direction of Madera et al. (1959) investigated the biological oxidation of TNT by activated sludge during digestion of the sludge. They incubated α-TNT (ranging from 5-50 mg/ℓ) with various concentrations of raw and digested activated sludge, and found that the majority of the TNT disappeared fairly rapidly. The metabolism of TNT was reported to have gone to completion via pathways similar to those reported for TNT breakdown in animals (Channon et al., 1944; see Section III-B). A two-stage model waste water purifier consisting of an aerator (1st stage), which was inoculated with Azotobacter agilis (reasons for using this organism are unclear), and a 2nd stage overflow basin which was inoculated with conventional activated sludge, was used by Bringmann and Kuehn (1971) to study the biological decomposition of a synthetic waste water which contained nitrotoluenes and nitrobenzenes (118 - 146 mg/ℓ). It was revealed in this study that dinitro- and trinitrotoluenes (2,4,6-trinitrotoluene, 2,4-dinitrotoluene, 2,6-dinitrotoluene) were removed to the extent of 95-97% after the 2nd stage; the mononitrotoluenes were practically completely removed. Only in the case of di- and trinitro-substituted compounds were small quantities of the reduced metabolites found to be present in the overflow from the aeration stage. Since only a moderate volume of oxygen was consumed, it appeared probable that some of the material was removed by absorption.

The results of the BOD tests performed by Nay et al. (1974) on pure α-TNT and TNT waste water resulting from the counter current-continuous flow TNT manufacturing process revealed that TNT was oxidizable at slow rates. The authors noted that mixing was very important to aid in keeping TNT in solution and in contact with the seed microorganisms. Furthermore, the TNT:microorganisms ratio had to be below the toxic level. The oxygen consumption was extremely concentration dependent and decreased considerably as the TNT loading increased. Nay (1972) performed another series of BOD tests in which the BOD water was supplemented with glucose; the increase in BOD was considered to be due to TNT consumption. The results of these studies also confirmed the oxidizability of TNT (Table 41). The authors' efforts to relate the BOD to the TNT removed were unsuccessful (Table 42). As can be seen, for approximately the same amount of TNT removed, the BOD values varied considerably. The authors suggested that the dilution factor was of significant importance in controlling their experiment. A likely explanation may be that higher TNT concentrations are toxic to certain TNT oxidizing microorganisms, and thus TNT may undergo only incomplete oxidation.

Table 41. Results of the Biodegradability Test on TNT and TNT Waste in Combination with Ammunition Plant Domestic Waste and Glucose.* (Nay, 1972)

	Concn. of TNT (mg/ℓ)	Waste Neutralization Procedure	16 Day BOD** Attributed to TNT (mg/ℓ)	TNT Removal (based on TNT Analysis) (%)
A. TNT Waste	18.5	Slowly with lime	71	42.7
	"	Slowly with soda ash	52	43.2
	"	Rapidly with soda	43	45.4
B. α-TNT	12.4	Slowly with lime	Less than control	0.0
	16.3	Slowly with soda ash	6	65.6
	20.2	Rapidly with soda ash	59	32.7

* Glucose equivalent to 5 mg COD, and domestic waste 50 ml (average composition/50 ml: C, 5.5 mg; N, 1.18 mg; P, 0.175 mg) per total test volume of 157 ml.

** Seed: Settled mixed liquor suspended solids from the bench scale continuous flow pilot plant that had been acclimated to Redford Army Ammunition Plant waste.

Table 42. Effect of TNT Concentration on the Biodegradability of TNT-Waste. (Nay, 1972)

TNT Concn. in Diluted Waste (mg/ℓ)	5 Day BOD** (mg/ℓ)	TNT Removed Based on TNT Analysis mg/ℓ	%
1	450	0.9	90.0
3	190	0.8	26.6
8	48	0.7	8.8
16	36	1.0	6.3

* Seed: Settled secondary influent of the Redford Army Ammunition plant trickling filter treatment plant.

** BOD test data cannot be expressed in terms of % COD or TOC since the COD value exerted per mg/ℓ TNT oxidized varied considerably from sample to sample, and with the initial concentration of TNT in the sample.

Following the BOD testing, Nay et al. (1974) evaluated the biological treatability of TNT waste in static tube and continuous flow runs. In the treatability experiments, TNT waste was diluted with the Army Ammunition plants domestic waste. The experimental findings from the static tube runs indicated that biological treatment can oxidize TNT from the waste at slow rates. The food to microorganisms ratio near 0.4 was found to be more amenable to biological treatment. The authors noted that biosorption or bioprecipitation of the TNT waste was much faster than the rate of oxidation of the waste. The biological treatment was ineffective in removing the color of the waste. In the continuous flow treatability runs, the activated sludge acclimated to TNT-waste for 10 days was used as inoculum. The treatability runs were made using 5 different concentrations of TNT (5-25 mg/ℓ); for each concentration tested, three different detention times were investigated to determine the effect of different organic loading rates. The average TNT removal efficiency for all 15 runs was nearly 65%, and the removal efficiencies showed a tendency to decrease with a decrease in detention time and increase in TNT concentration. From these findings, the authors concluded that TNT waste water can be biologically treated when combined with domestic waste. Although some TNT loss is evident from these studies, it should be emphasized, however, that a portion of TNT loss was due to biosorption on the activated sludge microorganisms and not due to molecular transformation.

In summary, the reports concerning biodegradability of nitrotoluenes are conflicting. There is no doubt that certain microorganisms possess the ability to alter trinitrotoluene and related compounds. No organisms have, however, been shown to use TNT as sole source of carbon and/or nitrogen. Degradation appears to occur by a cometabolic process. The majority of the organisms capable of degrading TNT were noted to be *Pseudomonas*-like. The experimental findings regarding biological treatability of the waste waters resulting from TNT manufacturing operations revealed that TNT-waste water can be biologically treated when combined with domestic waste.

(d) Routes of Degradation of Nitrotoluenes

The biochemical mechanism and pathway of TNT degradation by microorganisms are not well established. Won *et al*. (1974). reported identification of 2,2',6,6'-tetranitro-4-azoxytoluene, its isomer 2,2',4,4'-tetranitro-6-azoxytoluene, 4,6-dinitro-2-aminotoluene, 2,6-dinitro-4-hydroxylaminotoluene, nitrodiaminotoluene and trace quantities of the 2-amino- and the 4-amino-compounds in the cultures of *Pseudomonas*-like organisms (referred to as isolate Y) upon incubation with TNT. Based on their experimental findings, the authors proposed the following pathway for TNT metabolism (Figure 22).

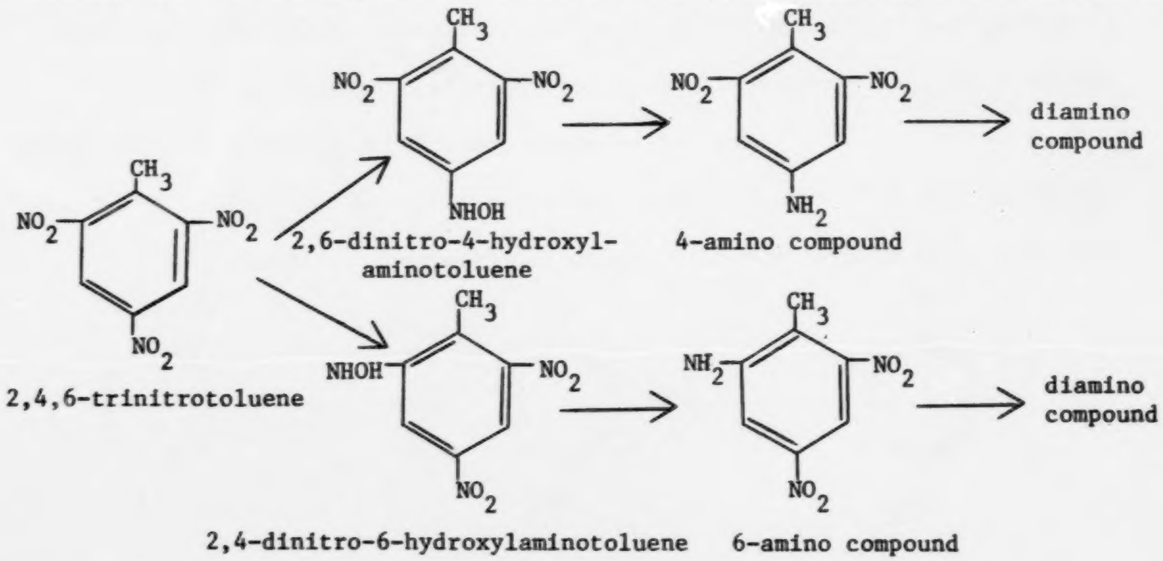


Figure 22. Proposed Pathway for TNT Metabolism
(Won *et al*., 1974)

The azoxy compound may not be the product of direct TNT metabolism as proposed by Channon et al. (1944) in the mammalian systems.

(v) Nitroanilines

A number of pure culture and mixed culture studies dealing with the decomposition of nitro-substituted anilines have been published. Salient features of these studies are presented in Table 43. The isolation of microorganisms active in decomposing p-nitroaniline, from a biofilm and the activated sludge of a laboratory purification installation, has been reported by Udod et al. (1972). The microorganisms belonged to the genera Pseudomonas and Bacillus, and had the ability to utilize p-nitroaniline (up to a concentration of 250 ppm) as the sole source of carbon and nitrogen. The o-isomer was degraded to the extent of 85-90% at a concentration of 100 mg/ℓ. An increase in the concentration of nitroaniline resulted in a lag in the decomposition of nitroaniline. The mixed culture of microorganisms prepared by mixing the isolated microorganisms was successful in degrading p-nitroaniline even in saturated solutions (approximate concentration 2 g/ℓ).

The intermediate products of p-nitroaniline decomposition were identified as p-phenylenediamine and p-aminophenol. The pathway for their formation is shown in Figure 23 (Udod et al., 1972).

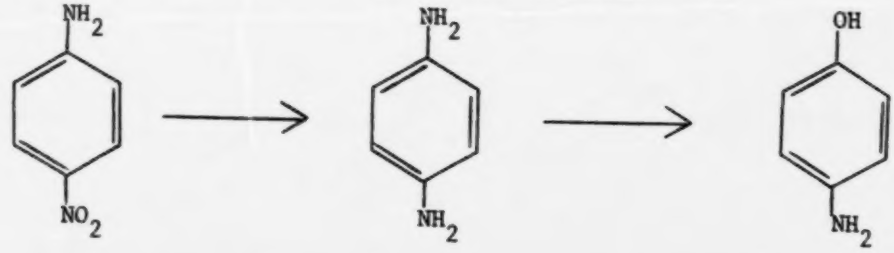


Figure 23. Intermediate Products of p-Nitroaniline Decomposition (Udod et al., 1972)

Table 43. Summary of the Degradation Studies with Nitroanilines

Reference	Test Chemical	Concn. Used	Source of Microorganisms	Duration of the Test	Criteria for Test Chemical Alteration
Malaney, 1960	*o*-, *m*-, and *p*-Nitroaniline	500 mg/ℓ	Aniline-acclimated activated sludge	8 Days	Oxygen uptake by Warburg Method
Chambers *et al*., 1963	*m*- and *p*-Nitroaniline	100 mg/ℓ	Microorganisms in soil compost or mud from a catalytic cracking plant waste lagoon, adapted to degrade phenol	210 Min.	Oxygen uptake by Warburg Method
Udod *et al*., 1972	*p*-Nitroaniline	100-500 mg/ℓ	Microorganisms isolated from a biofilm and the activated sludge of a laboratory purification installation	12-24 Hours	Growth, loss of the parent compound
Villeret, 1965	*m*-, *p*-, and *o*-Nitroaniline	0.69-.38.0 mg/ℓ	Fresh water alga *Chlorella vulgaris*	--	Growth with nitroaryl compounds as the nitrogen source
Alexander and Lustigman (1966)	*m*-, *p*-, and *o*-Nitroaniline	5-10 mg/ℓ	Soil	64 Days	Loss of U.V. absorbancy

The degradation products beyond *p*-aminophenol were not characterized in this study. However, since the cells were able to utilize *p*-nitroaniline for growth, it appeared that *p*-aminophenol underwent further degradation.

A number of investigators have studied the biodegradability of nitroanilines using a culture which has been adapted to degrade a compound similar in chemical structure to nitroaniline. Malany (1960) reported slow oxidation of *o*-, *m*-, and *p*-nitroaniline by the activated sludge which had been acclimated to aniline as sole source of carbon and energy. The susceptibility to oxidation decreased in the order *m*-, *p*-, *o*-. Chambers *et al*. (1963) reported rapid oxidation of *m*-nitroaniline by a culture which had been adapted to degrade phenol. The *p*-isomer was also attacked by these microorganisms, however, at a considerably slower rate.

The utilization of the nitro and amino groups of nitroanilines as the source of nitrogen for growth by fresh water algae *Chlorella vulgaris* was reported by Villeret (1965). The author found that the *meta*-isomer supported better growth than the *para*-isomer, with *ortho*-isomer giving minimum growth. No effort was made in this study to identify the transformation products.

The information presented here tends to suggest that nitroanilines are susceptible to rapid degradation by microorganisms. The *p*-isomer has been reported to be utilized as the sole source of carbon and nitrogen by certain microorganisms belonging to the genera *Pseudomonas* and *Bacillus*. The microorganisms capable of utilizing *o*- or *m*-isomer as the sole source of carbon and nitrogen have not been reported. This may be interpreted to mean that the *p*-isomer is relatively more biodegradable. On the other hand, the oxygen consumption studies with various isomers of nitroaniline as substrate suggest decreasing susceptibility to degradation in the order m-, p-, o-. The information available concerning the biodegradability of the various isomers of nitroaniline is conflicting and does not permit definite conclusions to be drawn regarding the order of their susceptibility to microbial attack.

(vi) Summary of the Biodegradation Studies With Nitroaromatics

A large number of nitroaromatic compounds have been tested for biodegradation. The major groups of compounds tested are nitroanilines, chloronitro- and nitrobenzenes, nitrobenzoic acids, nitrophenols, and nitrotoluenes. A variety of sources of seeds have been used by researchers in these studies. Some of the major sources are sewage, soil, river water, and pure cultures. However, very rarely in the biodegradation studies have researchers attempted to simulate other media than water. Although chemicals are first released into one specific medium, they often move from one medium to another. For example, nitrotoluenes usually pass through sewage treatment plants; however, if they are not degraded and/or removed there, they will contaminate the natural waters. It then becomes important to examine the persistence of nitrotoluenes in river water. The absence of information concerning the persistence of nitroaromatic compounds in a variety of media imposes a limit on the conclusions that can be drawn regarding the environmental fate of these chemicals.

The available information on the biodegradability of nitroaromatics is summarized in Table 44. Since all the compounds have not

Table 44. Biodegradability of Nitroaromatic Compounds under Varying Test Conditions.

Compound	Seed	Test Media	Relative Biodegradability
Nitroanilines			
m-Nitroaniline	AS, S	A	+ +
o-Nitroaniline	AS	A	+ +
p-Nitroaniline	AS, S, EP	A	+ + +
Chloronitro- and Nitrobenzenes			
o-Chloronitrobenzene	S, RW, SW	A	−
p-Chloronitrobenzene	S, EP	A	−
m-Dinitrobenzene	S, P, AS	A, SW	+
o-Dinitrobenzene	S, P	A	+
p-Dinitrobenzene	S, P	A	+
2,4-Dinitrochlorobenzene	S, EP	A	−
Nitrobenzene	S, P, AS, EP	A, SW	±
1,3,5-Trinitrobenzene	P, S, AS	A, SW	±
Nitrobenzoic Acids			
2-Bromo-4-nitrobenzoic acid	EP	A	+
2-Chloro-4-nitrobenzoic acid	EP	A	+
3,4-Dinitrobenzoic acid	S, AS	A	−
2,4-Dinitrobenzoic acid	EP	A	+ + +
2,5-Dinitrobenzoic acid	S	A	−
3,5-Dinitrobenzoic acid	AS	A	−
2-Fluoro-4-nitrobenzoic acid	EP	A	+
3-Fluoro-4-nitrobenzoic acid	EP	A	+
3-Hydroxy-4-nitrobenzoic acid	EP	A	−
2-Hydroxy-4-nitrobenzoic acid	EP	A	−
2-Iodo-4-nitrobenzoic acid	EP	A	+
3-Methyl-4-nitrobenzoic acid	EP	A	+
m-Nitrobenzoic acid	S, AS, P, EP	A	±
o-Nitrobenzoic acid	S, RW, AS, P, EP	A	+ + +
p-Nitrobenzoic acid	S, RW, AS, P, EP	A	+ + +
2,4,6-Trinitrobenzoic acid	S, AS	A	−
Nitrocresols			
4, 6-Dinitro-*o*-cresol	EP	A	+ + +
2,4,6-Trinitro-*m*-cresol	S	A	+ +
Nitrophenols			
2-Chloro-4-nitrophenol	S	A	±
4-Chloro-2-nitrophenol	S	A	±
2,6-Dichloro-4-nitrophenol	S	A	±

Table 44. Biodegradability of Nitroaromatic Compounds Under Varying Test Conditions (Cont'd.)

Compound	Seed	Test Media	Relative Biodegradability
Nitrophenols (cont.)			
2,4-Dinitrophenol	S, EP	A	-
2,6-Dinitrophenol	S, EP	A	-
2,5-Dinitrophenol	EP	A	-
Dinitro-(sec)butyl phenol	EP	A	-
2-Methyl-4-nitrophenol	EP	A	-
m-Nitrophenol	S, EP	A	+ + +
o-Nitrophenol	S, EP	A	±
p-Nitrophenol	S, EP	A	+ + +
2,4,6-Trinitrophenol	S, EP	A	±
Nitroresorcinols			
2,4-Dinitroresorcinol	S, RW	A	+ +
2,4,6-Trinitroresorcinol	S, RW	A	-
Nitrotoluenes			
2,4-Dinitrotoluene	S, EP	A	+ +
p-Nitrotoluene	P	A	±
2,4,6-Trinitrotoluene	S, P, SW, EP	SW, A	+

Seed

SW	Sewage seed
AS	Activated sludge
RW	River water
S	Soil
P	Stocked pure culture
EP	Enriched pure culture

Relative Biodegradability

Susceptible (extensive degradation, ring cleavage)	+ + +
Moderate (partial degradation, slow degradation)	+ +
Minor molecular alteration	+
Resistant	-
Uncertain	±

Test Media

S	Soil
A	Aqueous
SW	Sewage treatment

been tested under similar conditions, the biodegradability information provided in the table is of qualitative significance only. One must also keep in mind that the order of biodegradability shown in the table is under the conditions of the laboratory test; whether the relative biodegradability will be similar under environmental conditions is questionable.

2. Environmental Transport

Little experimental data are available concerning the extent and mechanism of transport of nitroaromatics in the environment. In the absence of this information, the environmental transport consideration of the nitroaromatics has been based primarily on the physical and chemical properties of these compounds.

a. Volatility

(1) Volatilization From Water

Organic chemicals are gradually lost to the atmosphere from aqueous solutions by codistilling with water. The volatility of a compound is dependent on its vapor pressure (which varies with temperature), water solubility, and adsorption properties (Kenaga, 1972). The amount volatilized increases with concentration until the maximum water solubility is reached. Mackay and Wolkoff (1973) have derived equations to predict the rate of evaporation of compounds from aqueous solutions using the water solubility and vapor pressure of the compound. In predicting the residence times, the authors assume that the water column undergoes continuous mixing and that the compound is present in the solution and not adsorbed, complexed, etc. Using this approach the half lives for certain nitroaromatic compounds have been calculated and are presented in Table 45. Unfortunately, the solubility and vapor pressure data

Table 45. Rate of Evaporation of Nitroaromatics from Bodies of Water (Calculated According to MacKay and Wolkoff, 1973).

Compound	Solubility* (mg/ℓ)	Vapor Pressure* (mm Hg)	M.W.	Calculated Half Life** at Ambient Temp. (approximate values)
Nitroanilines				
2-Nitroaniline	-	1^{104°	138	-
3-Nitroaniline	-	1^{119°	138	-
4-Nitroaniline	-	1^{142°	138	-
Nitrobenzenes				
Mononitrobenzene	1000^{20°	$1^{44.4^\circ}$	123	<8.8 Days
Nitrophenols				
o-Nitrophenol	3200^{38°	$1^{49.3^\circ}$	139	<25 Days
Nitrotoluenes				
p-Nitrotoluene	$40^{15^\circ}_{30^\circ}$	$1^{53.7^\circ}$	137	<44.6 Minutes
o-Nitrotoluene	652_{30°	1^{50°	137	<12.3 Hours
m-Nitrotoluene	500_{20°	$1^{50.2^\circ}$	137	<9.45 Hours
2,4,6-Trinitrotoluene	130	0.053^{85°	227	-
Other Compounds for Comparison				
DDT	12×10^{-3}	$1 \times 10^{-7^{25^\circ}}$	354.5	317 Days
Benzene	1780	95.2^{25°	78	37.3 Minutes

* Data from Matsuguma, 1967a; Nay, 1972. The superscripts after the solubility and vapor pressure refer to the temperature of measurement.

** At less than saturation concentration in a square meter of water.

are not available for the same temperature. For Table 45, half lives have been calculated based on the values of the two parameters at the available temperature, and from the anticipated variation of solubility and vapor pressure with temperature.

The experimental data on the loss of α-TNT from solution due to aeration has been obtained by Nay (1972). He conducted an air stripping experiment on both raw and neutralized waste samples. Incubations were performed in the absence of activated sludge to exclude the losses due to biological degradation. The results of this study revealed that only a small portion of the TNT concentration (about 8-10%) was vaporized during the experimental period of 18 days. The low evaporation rate of α-TNT into the atmosphere is supported by the fact that TNT has an extremely low vapor pressure (Table 45).

(ii) Volatilization From Soil and Other Surfaces

Volatilization of chemicals from soil and other surfaces is dependent upon the vapor pressure of the chemical as modified by the adsorptive interactions with the surface (Spencer and Cliath, 1975). The extent of reduction in vapor pressure is dependent upon the nature of the chemical, its concentration, water content of the soil, and soil properties (see Spencer _et_ _al_., 1973). It has been reported that chemicals in soil water are more easily lost than those that are adsorbed on soil particles (Huang, 1970).

Experimental data regarding the loss of pentachloronitrobenzene (PCNB, a fungicide) from soil has been obtained by Wang and Broadbent (1972). The authors found that the fungicide was lost from three

California soils (differing in their properties) mainly through volatilization. The calculated half times for PCNB in three soils were in the range of 4.7 - 9.7 months. Higher organic matter was associated with slower PCNB loss. Considering the solubility increasing effect of the loss of chlorines from the molecule, it can be predicted that the lower substituted chloronitrobenzenes will be lost more easily than pentachloronitrobenzene.

b. Leaching and Downward Movement of Nitroaromatics

The leaching potential of a chemical from soil depends on the extent to which it is adsorbed and its water solubility (see Browman and Chesters, 1975). The behavior of pentachloronitrobenzene in soil has been experimentally determined by Ko and Lockwood (1968). The authors reported that about 75% of the PCNB was still retained in natural soil (50% by soil, and 25% by soil organic matter and microorganisms) after three extractions (each for two days) with excess water. From these observations, it can be concluded that PCNB is unlikely to be lost by leaching. Considering that the loss of chlorine from the molecule tends to decrease its hydrophobicity and subsequently its retention by soil, it can be predicted that the lower halonitrobenzenes will be relatively more susceptible than PCNB to losses by leaching.

The factors governing the movement of a chemical to the ground water are the nature of the compound, its concentration and solubility in water, the composition and pH of the soil, the amount of rainfall and the height of the water table (see Browman and Chesters, 1975). The pertinent information needed to evaluate the ground water movement of nitroaromatics could not be found in the literature. The fairly high water solubility of many nitroaromatics suggests that these compounds will migrate through the soil and eventually contaminate ground water. The sorption of certain nitro compounds to clay and organic matter will tend to reduce their ground water movement. For example, Yariv and coworkers (Saltzman and Yariv, 1975, Yariv et al., 1966) have reported that nitrobenzene and nitrophenol can enter the inter layer space of the cation-saturated montmorillonite and form hydrogen bonds with water molecules in the hydration shell of the highly polarizing exchangeable cation.

c. Mobility in Water

Generally, the monitoring data provide a good indication of the mobility of a chemical in the aquatic environment. Unfortunately, very little monitoring information is available for nitroaromatic compounds. One of the chemicals, o-chloronitrobenzene has been found to travel long distances in surface waters; the compound was followed down the Mississippi River from St. Louis to the Gulf of Mexico (about 900 miles) at a concentration that could be simply explained by dilution (Kramer, 1965). This observation suggests only minimum adsorption to river sediment and a fairly high mobility of o-chloronitrobenzene in the aqueous environment.

Information regarding the mobility of other nitroaromatic compounds is not available. However, it may be possible to predict their mobility by considering the factors which affect the mobility of chemicals in the aqueous environment. Some of the important considerations in this respect are: adsorption of chemicals on hydrosoil or other particulate matter (those adsorbed will not be easily lost), water solubility, losses due to evaporation or degradation, etc. The nitro-substituted aromatic compounds dealt with in this report are fairly water soluble, and will most likely be contained in water. Subsequently, they will be expected to be freely mobile. However, a number of these chemicals may not persist long enough to be transported long distances. For example, mononitro-substituted benzoic acids (except the m-isomers) have been shown to be easily attacked by microorganisms. Nitrotoluenes are hard to degrade, but since they codistill fairly rapidly from water (except for α-trinitrotoluene), some losses may occur prior to their transport by water. Unlike these compounds, o-nitrophenol has a long calculated half life in water and is extremely water soluble; it can be predicted that this compound will travel to a certain extent in the aqueous environment at undiminished concentrations (except reduction by dilution or degradation).

3. Bioaccumulation

The nitroaromatic compounds may be present in water in very low concentrations. However, organisms of the lower aquatic food chain may be able to increase the concentration by accumulation of these compounds from their surrounding environment by various processes including absorption, adsorption, ingestion (bioaccumulation). In the absence of experimental data regarding the bioaccumulation potential of nitroaromatics, the assessment of their bioaccumulation potential can be estimated from their physical and chemical properties. Accumulation of any molecule occurs when the molecule is selectively concentrated in biological material and is accumulated faster than it is eliminated. Most nitroaromatic compounds (except for p-nitrotoluene, α-TNT, and perhaps certain halonitrobenzenes) are fairly water soluble, and it is unlikely that they will be taken up to a significant extent by aquatic organisms. Furthermore, in the case of nitrobenzoic acid, the available information suggests that these compounds will be rapidly attacked by microorganisms and therefore are not likely to be around to be taken up by food-chain organisms.

The octanol-water partition coefficient has been used in many instances to assess the bioconcentration potential of chemicals. Neely _et al_. (1974) have noted a linear relationship between octanol-water partition coefficients of several chemicals and bioconcentration factors (ratio of the concentration between the organism and the exposure water) in trout muscle. The water-octanol partition coefficients of a number of nitroaromatics are available from the literature (Leo _et al_., 1971). Using the equation of the straight line of best fit derived by Neely and coworkers (1974), the bioconcentration factors for nitroaromatics have been calculated (Table 46). As can be seen, nitroaromatic compounds in general do not have high bioconcentration potential. Of the compounds listed in the table, chloro-substituted nitrobenzenes and nitrotoluenes may perhaps be of some concern. However, it should be pointed out that the calculated values do not take into account the loss of the compounds from the organism due to metabolism and other mechanisms, so that the calculated value for a chemical will generally be higher than that determined experimentally as noted by Neely _et al_. (1974). Thus, it appears unlikely that nitrotoluenes or lower isomers of chloronitrobenzenes will bioaccumulate to a significant extent.

Further support for relatively low bioconcentration potential of the lower isomers of chloro-substituted nitrobenzenes comes from the data of Ko and Lockwood (1968). These investigators were able to show little accumulation and concentration above ambient levels of pentachloronitrobenzene in the mycelia of fungi. Incubation (48 hours) in soil containing 42 μg of PCNB

Table 46. Bioconcentration Factor of Nitroaromatics in Trout Muscle. (Calculated according to Neely *et al*., 1974)

Compound	Log, Octanol-Water Partition Coefficient*	Calculated Bioconcentration Factor (Ratio of the Concentration between the Organism and the Exposure Water)
Nitrobenzenes		
Nitrobenzene	1.85	13.37
4-Chloro-1-nitrobenzene	2.41	26.92
3-Chloro-1-nitrobenzene	2.41	26.92
2-Chloro-1-nitrobenzene	2.24	21.78
m-Dinitrobenzene	1.49	8.531
o-Dinitrobenzene	1.58	9.550
p-Dinitrobenzene	1.46	8.222
Nitrophenols		
m-Nitrophenol	2.0	16.13
o-Nitrophenol	1.79	12.42
p-Nitrophenol	1.91	14.46
2,4-Dinitrophenol	1.51	8.399
2,5-Dinitrophenol	1.75	11.81
2,6-Dinitrophenol	1.25	7.603
3,5-Dinitrophenol	2.32	24.04
Nitroanilines		
m-Nitroaniline	1.46	8.222
o-Nitroaniline	1.79	12.42
p-Nitroaniline	1.19	5.862
Nitrobenzoic Acids		
m-Nitrobenzoic acid	1.66	10.54
o-Nitrobenzoic acid	1.31	6.823
p-Nitrobenzoic acid	1.85	13.37
Nitrotoluenes		
m-Nitrotoluene	2.45	28.25
o-Nitrotoluene	2.30	23.45
p-Nitrotoluene	2.37	25.59
Other Compounds for Comparison		
Endrin	5.6	2953 ± 10
Benzene	2.13	19.0

* Data from Leo *et al*., 1971

per gram of moist soil led to the accumulation of PCNB in mycelia about 7 fold. Since reducing the number of chlorines on a molecule tends to reduce its hydrophobicity, which in turn is presumed to reduce its bioaccumulation potential, it can be predicted that less chlorine-substituted chloronitrobenzenes will be bioconcentrated to a lesser extent, if at all, than PCNB.

4. Biomagnification

Biomagnification refers to concentration of a compound through the consumption of lower organisms by higher organisms with net increase in tissue concentration (Isensee _et al._, 1973). The only reported study dealing with the biomagnification of nitroaromatic compounds is that of Metcalf and Lu (1973). The authors studied the ecological magnification of nitrobenzene in a model aquatic ecosystem which permitted passage of the chemical through the aquatic fauna and flora. The findings revealed low overall biomagnification of nitrobenzene in fish (biological magnification* = 20; for comparison biological magnification for DDT under similar conditions = 16,950). Most of the radioactivity present in the fish was in the form of _p_-nitrophenol and its conjugate. Knowing the biomagnification potential of nitrobenzene may enable one to predict the behavior of halogenated nitrobenzenes in the food chain. Since substitution of chlorine on nitrobenzene will presumably increase its hydrophobicity, it appears reasonable to conclude that halonitrobenzenes will have a greater biomagnification potential.

For other nitroaromatic compounds, their water solubility may also be helpful in predicting their biomagnification potential. Metcalf and Lu (1973) have reported a straight line relationship between ecological magnification of several chemicals in their model aquatic ecosystem, and the water solubility. Using this relationship, the ecological magnification values for several nitroaromatics have been calculated (Table 47).

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* concentrations in organism/concentration in water

In summary, the solubility characteristics of nitroaromatic compounds, in general, indicate that they will not biomagnify or bioaccumulate to a significant extent in the food chain. However, it is likely that certain halonitroaromatics may biomagnify since chlorine substitution will tend to make them more hydrophobic.

Table 47. Ecological Magnification in the Model Aquatic Ecosystem for Nitroaromatics (Calculated According to Metcalf and Lu, 1973).

Compound	Solubility at 20°C (mg/ℓ)	Calculated Ecological Magnification in Fish at Ambient Temperature (approximate values)
o-Nitrophenol	3.2×10^{6} $^{38^\circ}$	<2.7
p-Nitrophenol	$40.0^{15}_{30^\circ}$	>43
o-Nitrotoluene	652.0	<7.5
m-Nitrotoluene	500.0^{30°	<8.8
Nitrobenzene	1.51×10^{4}	78.9
4-Chloronitrobenzene	2.87×10^{3}	224
2-Chloronitrobenzene	2.80×10^{3}	227
3-Chloronitrobenzene	1.73×10^{3}	307
3,4-Dichloronitrobenzene	6.29×10^{2}	579
2,5-Dichloronitrobenzene	4.8×10^{2}	686
2,3-Dichloronitrobenzene	3.25×10^{2}	876
2,4,5-Trichloronitrobenzene	1.3×10^{2}	1555
2,3,4-Trichloronitrobenzene	1.15×10^{2}	1682
2,3,4,6-Tetrachloronitrobenzene	29.0	3987
2,3,4,5-Tetrachloronitrobenzene	28.0	4074
2,3,5,6-Tetrachloronitrobenzene	8.0	8933
2,3,4,5,6-Pentachloronitrobenzene	1.5	25520
DDT (for comparison)		16950

Data from Matsuguma, 1967a, and Eckert, 1962.

B. Biology

The biological activities of foreign organic compounds are determined in large part by their metabolic fate. Metabolic pathways have evolved in which foreign compounds are chemically transformed by oxidation, reduction, hydrolysis, and conjugation reactions into metabolites which are inactive, or which are more water-soluble and therefore more readily excreted. These metabolic pathways have been described as detoxication mechanisms, since foreign compounds seem to be converted to less toxic or inert products. However, in some cases, biologically active metabolites are produced.

Most biotransformations of foreign organic compounds are catalyzed by a series of enzymes present in any of several tissues and organs. By far, the greatest number of the enzymes of importance in foreign compound metabolism are in the liver; however, other sites include the kidney, the skin, the intestine, the lung, or the placenta.

While the transformations of most foreign compounds are accomplished by enzyme-catalyzed reactions, other factors influence their metabolism. These factors include the structure of the foreign compound, the route of absorption, storage in fat, binding to plasma proteins, localization in tissues, and sensitivity of target molecules. Metabolism may also be affected by diet and by the genetic makeup of the species.

1. Absorption and Elimination

Although exposure to most industrial poisons occurs by absorption through the respiratory tract, nitroaromatic derivatives are also readily absorbed through the skin. A large number of occupational and accidental poisoning cases have directly resulted from dermal exposure to nitroaromatic chemicals (see Section III-C). Subsequent to skin absorption, localization and storage of these compounds in the body fat often occurs. The stored chemical can be readily mobilized by ingestion of alcohol or exposure to sunlight (Rejsek, 1947), resulting in an episode of serious intoxication.

The relationship between toxicity and mode of absorption for the nitroaromatics is largely dependent upon their molecular structure, lipid solubility, and degree of ionization. As a group, the nitroaromatic compounds are readily soluble in organic solvents and therefore can usually penetrate the intact skin with ease. Passive diffusion across the skin and other lipoidal membranes is greatest for lipid-soluble neutral molecules (i.e., undissociated compounds having a high lipid/water partition coefficient). Absorption and rate of passive diffusion decrease with increasing ionization, as illustrated in Table 48. The absorption of chemicals that can dissociate will be favored by a change in pH that increases the proportion of the undissociated form.

Table 48. Intestinal Absorption in Rats From Solutions of Various pH Values

- The percent absorbed is expressed as the mean ± the range. The figure in parentheses indicates the number of animals. -

(From Hogben *et al*., 1959)

Drug	pKa	Per Cent Absorbed			
		pH of the intestinal solution			
		3.6-4.3	4.7-5.0	7.2-7.1	3.0-7.8
Bases					
Aniline	4.6	40 ± 7 (9)	48 ± 5 (5)	58 ± 5 (4)	61 ± 9 (10)
Aminopyrine	5.0	21 ± 1 (2)	35 ± 1 (2)	48 ± 2 (2)	52 ± 2 (2)
p-Toluidine	5.3	30 ± 3 (3)	42 ± 3 (2)	65 ± 4 (3)	64 ± 4 (2)
Quinine	8.4	9 ± 3 (3)	11 ± 2 (2)	41 ± 1 (2)	54 ± 5 (5)
Acids					
5-Nitrosalicylic	2.3	40 ± 0 (2)	27 ± 2 (2)	<2 (2)	<2 (2)
Salicylic	3.0	64 ± 4 (4)	35 ± 4 (2)	30 ± 4 (2)	10 ± 3 (6)
Acetylsalicylic	3.5	41 ± 3 (2)	27 ± 1 (2)	-	-
Benzoic	4.2	62 ± 4 (2)	36 ± 3 (4)	35 ± 4 (3)	5 ± 1 (2)
p-Hydroxypropiophenone .	7.8	61 ± 5 (3)	52 ± 2 (2)	67 ± 6 (5)	60 ± 5 (2)

Nitroaromatic chemicals which act as weak acids or weak bases will often be incompletely ionized at physiological pH. The degree of ionization will depend on both the physiological pH and the pKa value for the particular chemical. The absorption of several nitroaromatic compounds and other organic electrolytes from rat small intestine was shown to be related to their pKa values as illustrated in Tables 49 and 50. Acidic compounds having pKa values greater than 3 and basic compounds with pKa values less than 8 were rapidly absorbed.

Table 49. Absorption of Organic Acids From the Rat Small Intestine - The percent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses (From Schanker *et al.*, 1958) -

Acid	pKa	Per Cent Absorbed	
		Actual	Relative to aniline
5-Sulfosalicylic	(strong)	<2 (2)	<2
Phenol red	(strong)	<2 (4)	<2
Bromphenol blue	(strong)	<2 (2)	<2
o-Nitrobenzoic	2.2	5 ± 2 (2)	5
5-Nitrosalicylic	2.3	9 ± 2 (3)	9
Tromexan	2.9	35 ± 7 (3)	37
Salicylic	3.0	60*	--
m-Nitrobenzoic	3.4	53 ± 0 (2)	50
Acetylsalicylic	3.5	20 ± 4 (6)	21
Benzoic	4.2	51 ± 5 (2)	54
Phenylbutazone	4.4	65 ± 7 (3)	54
Acetic	4.7	42 ± 1 (3)	40
Thiopental	7.6	55 ± 6 (3)	67
Barbital	7.8	30 ± 4 (2)	25
p-Hydroxypropiophenone	7.8	61 ± 9 (5)	61
Phenol	9.9	51 ± 8 (3)	60

* Standard deviation, ± 10 per cent for 30 experiments.

Table 50. Absorption of Organic Bases From the Rat Small Intestine

- The percent absorbed is expressed as the mean ± the range followed by the number of experiments in parentheses (from Schanker, et al, 1958) -

Base	pKa	Per Cent Absorbed	
		Actual	Relative to salicylic acid
Acetanilide	0.3	42 ± 5 (2)	43
Theophylline	0.7	29 ± 1 (3)	30
p-Nitroaniline	1.0	68 ± 7 (2)	61
Antipyrine	1.4	32 ± 6 (3)	30
m-Nitroaniline	2.5	77 ± 2 (2)	63
Aniline	4.6	54*	--
Aminopyrine	5.0	33 ± 4 (4)	27
p-Toluidine	5.3	59 ± 3 (3)	56
Quinine	8.4	15 ± 2 (6)	15
Ephedrine	9.6	7 ± 3 (2)	6
Tolazoline	10.3	6 ± 1 (2)	5
Mecamylamine	11.2	<2 (2)	<2
Darstine	(strong)	<2 (2)	<2
Procaine amide ethobromide	(strong)	<2 (2)	<2
Tetraethylammonium	(strong)	<2 (2)	<2
Tensilon	(strong)	<2 (2)	<2

* Standard deviation, ± 10 percent for 43 experiments.

The nitroaromatic compounds with a relatively high vapor pressure, particularly the nitrobenzenes, are quickly absorbed upon inhalation. The pulmonary epithelium, unlike most other body membranes, is easily penetrated by both lipid-soluble molecules and large lipid-insoluble molecules and ions (Enna and Schanker, 1969). This factor is often of greater practical importance in determining the hazards of environmental exposure than a simple comparison of toxicity data obtained by oral or parenteral administration of various substances.

a. Nitrophenol Derivatives

Several investigators have undertaken to study the absorption and elimination of nitroaromatic compounds by measuring their levels in the blood after administration by various routes. For the most part, these studies have involved the derivatives of dinitrophenol which are important as agricultural chemicals and, therefore, pose the greatest risk of exposure.

Parker _et_ _al_. (1951) investigated the fate of 4,6-dinitro-_o_-cresol (DNOC) in rabbits, cats, rats, and dogs after subcutaneous injection. Figure 24 shows the exponential drop in blood serum levels of DNOC after a single injection. It can be seen that a species variation exists in the rate of fall.

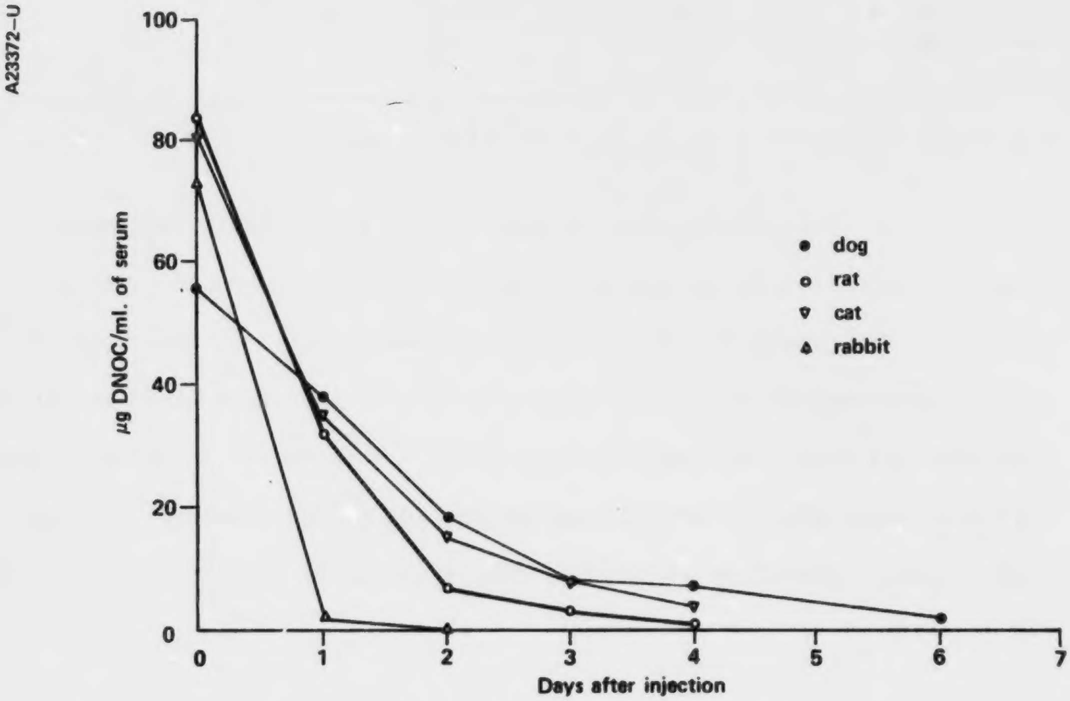

Figure 24. Changes in Serum Level of DNOC After a Single Subcutaneous Injection of 10 mg DNOC/kg (Parker _et_ _al_., 1951)

The administration of a series of daily subcutaneous injections in the rabbit did not change the rate of DNOC elimination from the blood, nor did it produce a cumulative rise in the blood level (Figure 25).

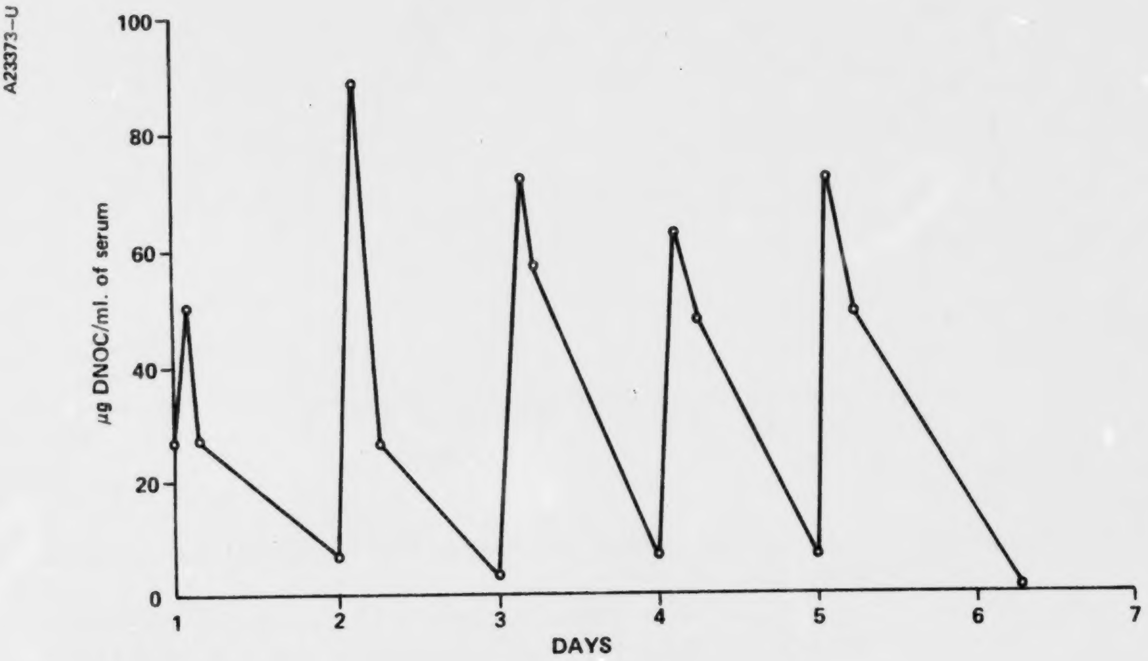

Figure 25. Effect of Repeated Subcutaneous Injections (10 mg DNOC/kg) on Serum Level of DNOC in Rabbit. Samples of Serum Analyzed Immediately After Injection and Then at One, Three, Five, and Twenty-four Hours (From Parker et al., 1951)

Similarly, in the rat and dog, blood levels of DNOC still fell to the same level after repeated daily injections, even though the rate of DNOC elimination by these species is slower than in the rabbit, as shown in Figure 24 above. In addition, Parker and his coworkers demonstrated that the rate of DNOC elimination from the blood was not altered by pre-treating the animal with a series of daily injections (Figure 26).

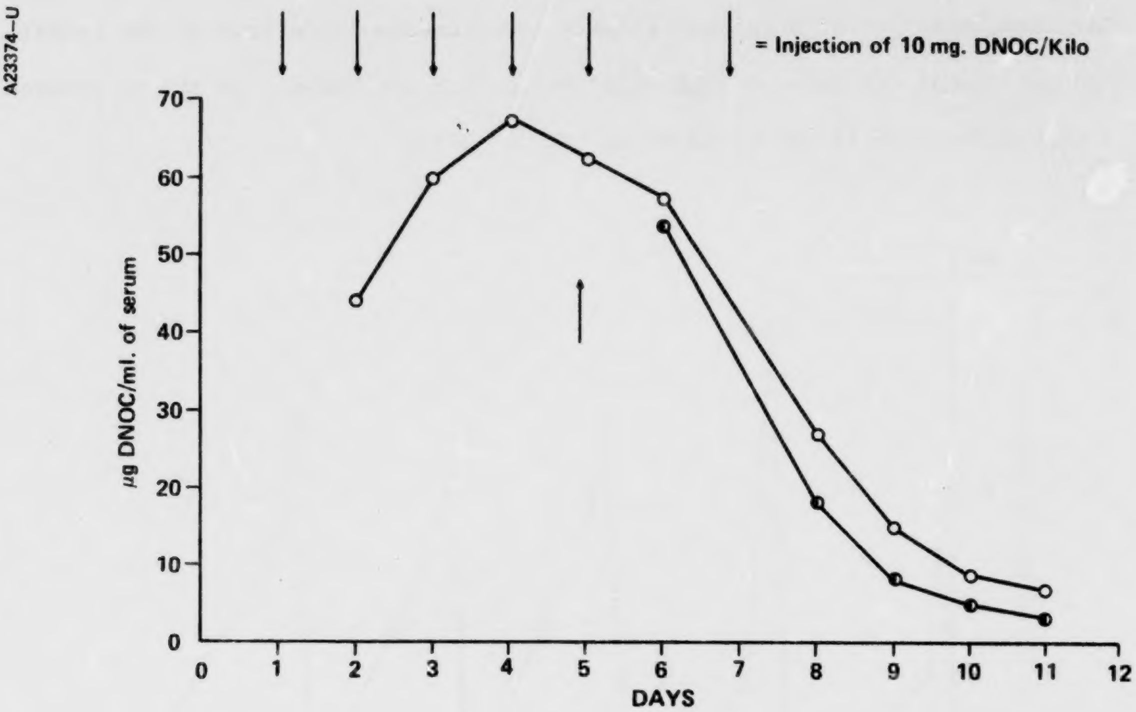

Figure 26. Blood Levels of DNOC in the Dog After Repeated Subcutaneous Injections

- The upper curve shows the effect of five daily subcutaneous injections on the DNOC concentration in serum of dog (serum samples taken 24 hours after the previous injection). After the fifth day, no further injections were given.
The lower curve shows the rate of fall of DNOC in serum following a single injection 24 hours before the first sample was taken. (From Parker et al., 1951)

Overall, there is clearly no evidence from these studies to indicate any likelihood for significant DNOC accumulation in the blood of laboratory animals.

Further observations on the absorption and elimination of DNOC were made possible through a number of animal studies conducted by King and Harvey (1953a, 1953b). Levels of DNOC in the blood were determined after its absorption through the gut, the lungs, and the skin of rabbits and rats. When given by stomach tube to rats, DNOC reached maximum blood levels within seven hours (Table 51). Large quantities of unchanged DNOC did not remain in the gut. One hour after dosing, 20% of the dose could be recovered from the gut, and after two hours, only about 10% remained unabsorbed.

Table 51. Absorption of DNOC Following Single Dose of 30 mg/kg Given by Stomach Tube to Albino Rats (King and Harvey, 1953 a) (Rats bled and then killed immediately by ether; entire gut removed; contents well washed out; tissues and contents homogenized before analysis.)

			DNOC (µg.)				Total DNOC	
Dose (mg.)	Time after dose (hr.)	Blood (µg./g.)	Stomach	Small intestine	Large intestine	Contents of alimentary canal	(mg.)	% of dose
4.14	1	34.0	110.0	13.0	11.0	660.0	0.79	19.2
6.00		30.0	292.0	10.0	5.0	800.0	0.11	18.5
4.62	2	60.5	55.0	16.3	20.0	450.0	0.54	11.7
5.01		45.5	61.0	13.0	8.0	540.0	0.62	12.4
4.65	4	36.5	22.0	13.0	7.0	92.0	0.13	2.9
4.62		29.3	26.0	12.0	6.0	124.0	0.17	3.6
4.89	7	57.2	33.0	13.0	26.0	114.0	0.18	3.8
3.60		52.0	33.0	13.0	12.0	18.0	0.07	2.1
4.86	24	22.5	5.0	11.0	Lost	8.0	>0.02	>0.5
3.90		25.0	6.0	11.0	17.0	6.0	0.04	1.0
3.45	48	3.0	1.0	4.0	12.0	7.0	0.02	0.7
3.66		2.8	1.0	3.0	14.0	7.0	0.02	0.7

Intraperitoneal injection versus administration by stomach tube of identical DNOC doses demonstrated that higher maximum blood levels could generally be attained by the parenteral route (Table 52). However, the time to reach maximum levels following the two treatments does not seem to be consistently shorter by either route, as shown in Table 52. On this basis, one can predict that DNOC is readily absorbed across the gut wall but may be partially degraded by intestinal contents, resulting in lower blood levels than can be achieved by injection of the same dose.

Table 52. Blood DNOC Levels in Animals Following Single Doses (From King and Harvey, 1953 a)

(ST, administration by stomach tube; IP, intraperitoneal injection. All animials survived dosage except two rats, 20 and 100 mg/kg. Each experiment was on a single animal except for the 30 mg/kg (two rats) and 40 mg/kg (twelve rats); for the latter group mean ± S.E. is given).

Dose (mg./kg.)	Admin.	DNOC (µg./g.)											
		Time after dose (hr.)											
		1	2	3	4	5	6	7	8	11	12	24	27
						Rats							
1	Oral	2.5	--	3.3	--	3.8	--	--	2.9	--	--	3.7	--
5	ST	8.8	--	13.0	--	9.3	--	--	--	--	--	12.0	--
10	ST	48.0	--	16.0	--	26.0	--	--	--	--	--	25.0	--
20	ST	46.0	--	17.0	--	48.0	--	--	--	--	--	--	--
30	ST	34.0	60.5	--	36.5	--	--	57.2	--	--	--	22.5	--
		30.0	45.5	--	29.3	--	--	52.0	--	--	--	25.0	--
40	ST	--	100.3±	--	100.5±	--	97.4±	--	--	--	--	--	--
			1.98	--	2.85	--	1.53	--	--	--	--	--	--
50	ST	--	--	58.0	60.0	--	70.0	--	92.0	--	80.0	19.0	--
100	ST	--	--	85.0	88.0	--	--	--	--	--	--	--	--
1	IP	4.7	--	7.1	--	5.0	--	--	4.9	--	--	--	--
5	IP	8.8	--	14.1	--	15.7	--	--	23.8	12.2	--	--	9.8
10	IP	28.4	--	40.5	--	33.7	--	--	34.7	30.6	--	--	15.1
20	IP	101.0	--	97.0	--	63.2	--	--	64.6	43.5	--	--	25.8
						Rabbits							
5	Oral	--	8.1	--	6.4	--	10.0	--	20.0	--	4.6	7.4	--
10	ST	--	24.0	--	44.0	--	20.0	--	18.0	--	5.5	4.0	--
20	ST	--	24.0	--	31.0	--	31.0	--	8.2	--	18.8	2.5	--

Evidence was obtained by King and Harvey indicating that accumulation of DNOC can occur to a limited extent in animals after repeated doses, either by stomach tube or by intraperitoneal injection. Blood level determinations for DNOC were made in rats and rabbits given the compound on a daily basis. Their results showed that a significantly higher DNOC blood level was achieved in some groups of rats after two daily doses, as compared to that resulting from a single dose. Increases in blood DNOC levels between days two and three, however, were not usually significant. The rabbit, on the other hand, did not appear to accumulate DNOC in any fashion from these experiments.

The results of inhalation studies demonstrated that absorption of DNOC can occur readily through the lungs and, in fact, prolonged inhalation can lead to death (Figure 27).

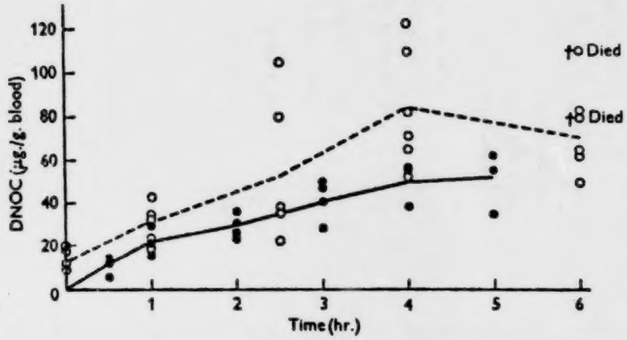

Figure 27. Effect of DNOC Aerosol on Rats; Blood DNOC Values After Exposure at 25° to Concentration of 0.1 mg/cu m

●——●, Four albino rats (not previously dosed
0-----0, Six hooded rats previously given sixteen daily doses of 5 mg/kg DNOC by intraperitoneal injection; dots and circles show values for individual rats; lines show mean value (King and Harvey, 1953 a)
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Absorption via the lungs was considered by the authors to be particularly dangerous to health, since small particles containing DNOC can be carried directly to the lung surface. Thus, DNOC would then be rapidly absorbed into the circulation without passing first through the liver. This route of absorption is far more efficient than exposures occurring through the gut or across the skin.

Determining the absorption of DNOC through the skin was complicated by several variables. Although increased environmental temperature produced a marked rise in the metabolic rate and increased mortality in dermally-treated rabbits, blood levels of DNOC were not apparently affected (Figure 28).

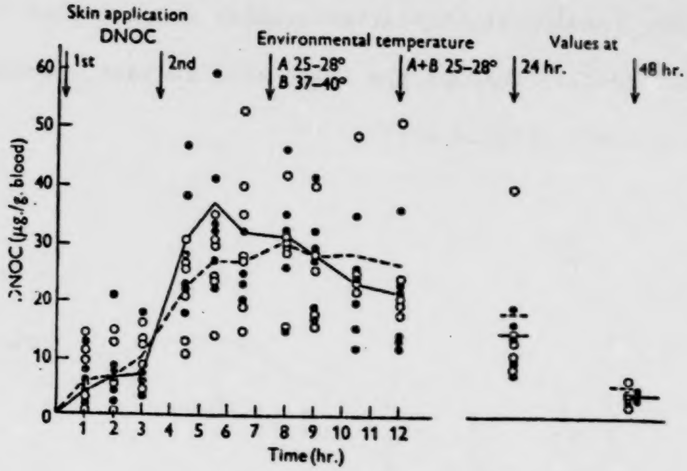

Figure 28. Skin Absorption of DNOC by Twelve Chinchilla Rabbits

2% aqueous (w/v) DNOC (as Na salt) to give 2 mg/sq cm over 50 sq cm. Group A, O-----O (broken line mean value); Group B, ●———● (continuous line mean value). Slopes from 8.0 to 12.0 hr. A: -2.05, +2.68, -1.50, -1.48, -1.65, -3.08; B: -2.42, -3.49, -2.35, -1.19, -2.26, -2.70. $d(X_A - X_B) = 1.79$, S.E. (d)=0.96 not significant. (From King and Harvey, 1953 a) (Reprinted from the Biochemical Journal with permission from the Biochemical Society.)

Clearly, however, DNOC was absorbed through the skin and, in fact, significant quantities remained in the blood (2.4 to 7.9 µg/g) even 48 hours after application of the treatment dose. This would suggest a possible storage or accumulation of DNOC in the skin which can result in the sustained release of small quantities into the blood for prolonged periods.

In a study employing human volunteers, Harvey et al. (1951) administered DNOC to five men in order to study its accumulation potential. The authors pointed out that, while DNOC and other dinitrophenol derivatives are not cumulative poisons in animals, occupational studies have indicated that DNOC may be accumulated in man (Bidstrup and Payne, 1951; see Section III-C).

The results of administering repeated oral doses of 75 mg of DNOC are presented in Figure 29.

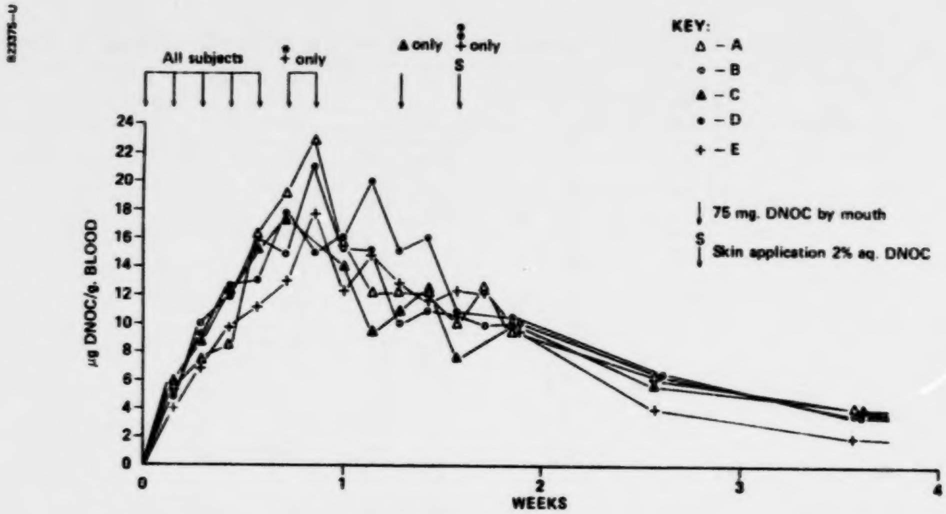

Figure 29. Comparison of 24-Hour Blood Levels (Harvey et al., 1951)

These experiments showed that DNOC remained in the blood at 1 to 1.5 µg/g for up to forty days after administration of the last oral dose. In addition, absorption through the skin was demonstrated, as well as the fact that exercise caused an increase in the concentration of DNOC in the blood. The latter observation may be indicative of binding to the albumin fraction of the blood. Levels in the blood of 15 to 20 µg DNOC per gram of blood were associated with symptoms of poisoning and corresponded to an absorption on the order of 1 mg per kg body weight of DNOC for three to five days. These results suggest that exposure to relatively low levels of DNOC, when continuing over a period of several days, may pose a serious threat to health.

In further studies on the retention of DNOC by man and animals, King and Harvey (1953 b) considered the problem of DNOC storage in the body and the capacity for man, rats, and rabbits to eliminate DNOC. A plot of the regression lines expressing the decay in blood DNOC levels for man, rat, and rabbit, calculated from the data in Table 53, is presented in Figure 30. These results show an exponential decay in all cases, but also demonstrate that man is relatively inefficient in eliminating DNOC.

Table 53. Decaying Blood DNOC Values in Man and in Animals (King and Harvey, 1953 b)

Species, number and sex	Daily dose of DNOC (mg/kg)	Time after dosing (hr)	Blood DNOC (μg/g) Mean±S.E.	Slope (b)	Half-time (hr)
Man (1)	--	--	See Pollard & Filbee, 1951	-0.002	153.6
Rat (F) (4)	9 x 20	6.0	72.2 ± 10.0	-0.0105	26.8
		24.0	50.7 ± 7.5		
		48.0	23.7 ± 4.9		
		72.0	17.1 ± 3.3		
		120.0	7.1 ± 1.5		
		144.0	2.0 ± 0.5		
Rat (F) (5)	1 x 30	3.5	105.0 ± 10.0	-0.0112	28.5
		24.0	64.6 ± 4.8		
		46.5	32.5 ± 4.0		
		72.0	19.3 ± 2.7		
		77.0	13.9 ± 1.7		
		95.0	11.2 ± 2.1		
Rabbit (F) (6)	9 x 25	4.5	54.7 ± 6.6	-0.0448	6.7
		7.5	44.4 ± 5.3		
		10.5	31.2 ± 3.1		
		24.0	6.1 ± 1.07		
		48.0	0.7 ± 0.2		
Rabbit (F) (6)	1 x 30	6.0	49.5 ± 3.4	-0.0454	6.6
		9.0	46.8 ± 2.9		
		12.0	30.9 ± 2.4		
		25.0	7.7 ± 1.3		
		31.0	4.2 ± 1.0		
		49.0	0.8 ± 0.3		

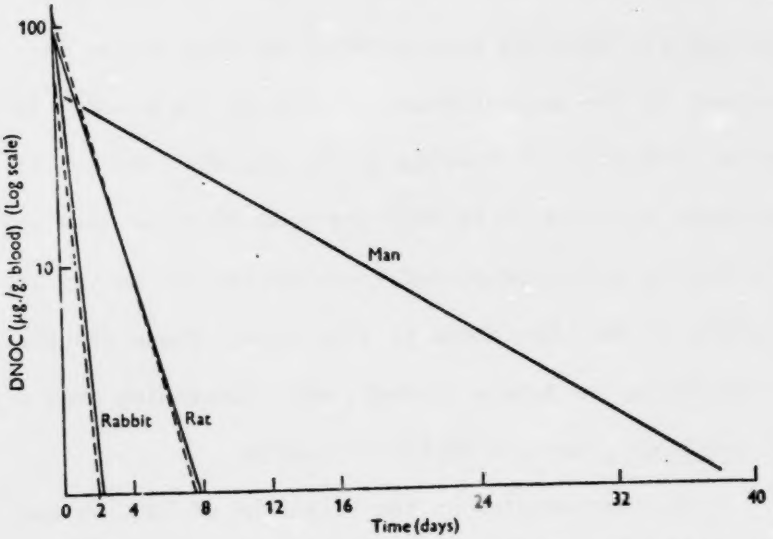

Figure 30. Decay Curves of Blood DNOC of Man, Rat, and Rabbit (King and Harvey, 1953 b)
(Continuous lines represent curves resulting from many doses; broken lines from a single dose.)
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Twenty-four hours after the administration of a single 75 mg dose of DNOC to human volunteers, only about 40 percent of the compound could be accounted for (Table 54).

Table 54. Distribution of DNOC in Man Following Single Dose of 75 mg DNOC (King and Harvey, 1953 b)

Volunteer subject	Blood volume (ml.)	Accountable DNOC 24 Hr. After Dose				DNOC which cannot be accounted for (% intake)
		In blood		In urine		
		(mg.)	(% intake)	(mg.)	(% intake)	
A	5200	28.7	38.2	0.8	1.1	60.7
B	5400	30.3	40.4	0.6	0.80	58.8
C	5800	35.0	46.6	1.3	1.7	51.7
D	5600	26.4	35.2	0.6	0.80	64.0
E	6000	26.7	35.5	1.5	2.00	62.5

This observation suggested that excretion of DNOC may be occurring very slowly, or that storage of DNOC in the body may have been taking place. However, it is not possible to determine from these results whether the non-accounted for fraction was present in the form of unchanged DNOC or as unidentifiable (and therefore non-accounted for) metabolites.

Further studies on the elimination of nitrophenolic compounds by various animals were conducted by Lawford _et al_. (1954). Determinations were made of the absolute slope values of the elimination rates from blood of four nitrophenol derivatives given to various animals. Table 55 details the rates of elimination following either oral or parenteral administration of _p_-nitrophenol, 2,4-dinitrophenol, DNOC, and 2,4-dinitro-α-naphthol.

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Table 55. Absolute Rates of Elimination(a) of Four Nitro-Compounds (Lawford et al., 1954)

Animal	Method of dosage	Substance mol. wt. solubility in water g./100 ml.	p-nitrophenol 139 1.6 (25°C)	2,4-dinitrophenol 184 0.56 (18°C)	4,6-dinitro-o-cresol 198 0.024 (19°C)	2,4-dinitro-α-naphthol 234 0.004 (18°C)
Mouse	Oral	S (a)	30	36	44	
		T	30	36	44	(c)
		b	- 0.90 ± 0.06	- 0.098 ± 0.033	- 0.036 ± 0.004	
	Intra-peritoneal	S	24	24	28	20
		T	24	24	28	20
		b	- 1.24 ± 0.12	- 0.21 ± 0.014	- 0.04 ± 0.002	- 0.012 ± 0.006
Rabbit	Oral	S	4	6	6	4
		T	64	24	30	16
		b	- 0.43 ± 0.036	- 0.010 ± 0.02	- 0.045 ± 0.001	- 0.061 ± 0.02
	Intra-peritoneal	S	5	6	3	4
		T	45	24	15	20
		b	- 0.78 ± 0.006	- 0.22 ± 0.0009	- 0.077 ± 0.0109	- 0.087 ± 0.02
Guinea-pig	Oral	S		16	16	20
		T	(b)	16	16	20
		b		- 0.12 ± 0.017	- 0.032 ± 0.001	- 0.051 ± 0.004
	Intra-peritoneal	S		16	20	16
		T	(b)	16	20	16
		b		- 0.135 ± 0.017	- 0.021 ± 0.003	- 0.04 ± 0.004
Rat	Oral	S	4	6		4
		T	32	24	- 0.01	24
		b	- 0.190 ± 0.012	- 0.062 ± 0.009		- 0.015 ± 0.0006
	Intra-peritoneal	S	5	6		4
		T	25	24	- 0.02	20
		b	- 0.80 ± 0.06	- 0.122 ± 0.0008		- 0.021 ± 0.0001

NOTES: (a) S = number of animals; T = number of blood samples. These were always arranged in equal groups. Thus, when S = 4 and T = 64, there were 16 equal sample groups. All mice and guinea-pigs gave one sample each so S = T. Groups were spaced farily evenly over time necessary for total elimination. This was determined approximately by preliminary experiments involving a small number of animals. (b) Values too scattered to give a satisfactory regression line.

The above data indicate that, of the four substances tested, the rates of elimination would decrease in the order p-nitrophenol, 2,4-dinitrophenol, 2,4-dinitro-α-naphthol, and DNOC. This relationship may also be seen in Figure 31, which depicts the rates of elimination from the blood for these four compounds when administered to the monkey.

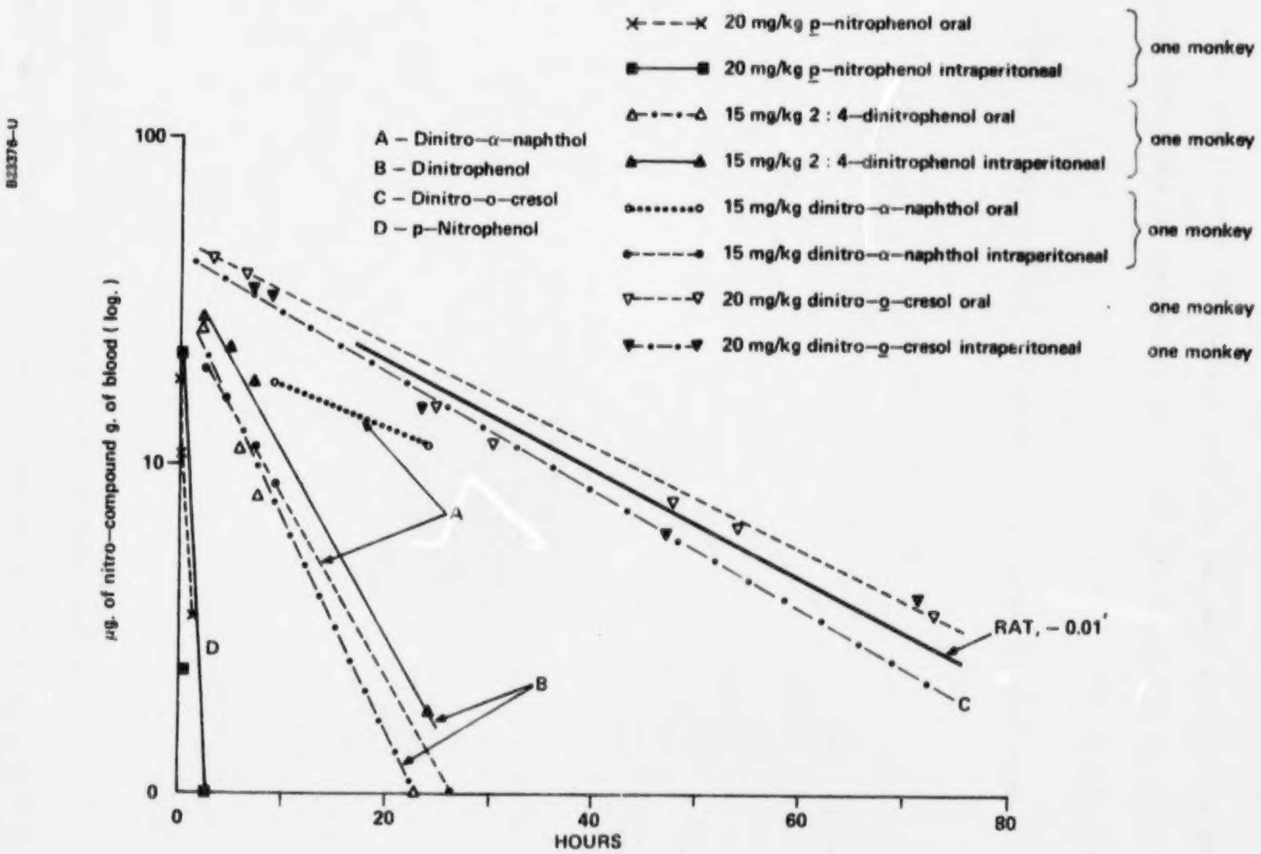

Figure 31. Elimination of Nitro Compounds By the Monkey (From Lawford et al., 1954)

The comparisons presented in Table 56 depict elimination rates expressed as ratios of the absolute slope values, with either the rat or DNOC = 1. These results indicate that in the four species of animals employed in this study, the ability to eliminate the chemicals decreased in the order mouse > rabbit > guinea pig > rat.

Table 56. Comparison of Rates of Elimination (Lawford et al., 1954)

(A) By Animal Species: Rat = 1

Substance		Mouse		Rabbit		Guinea-pig		Rat
p-nitrophenol	Oral	4.9	>	2.3	>	(N.A.)	>	1.0
	Intra-peritoneal	1.5	>	1.0	=	(N.A.)	=	1.0
2,4-dinitro-phenol	Oral	1.5	<	1.6	<	2.0	>	1.0
	Intra-peritoneal	1.7	<	1.8	>	1.1	>	1.0
4,6-dinitro-o-cresol	Oral	3.6	<	4.5	>	3.2	>	1.0
	Intra-peritoneal	2.0	<	3.8	>	1.0	=	1.0
2,4-dinitro-α-naphthol	Oral	(N.A.)		4.0	>	3.4	>	1.0
	Intra-peritoneal	6.0	>	4.1	>	1.9	>	1.0

(B) By Compounds: dinitro-o-cresol = 1

Animal		p-nitrophenol		2,4-dinitrophenol		2,4-dinitro-α-naphthol		4,6-dinitro-o-cresol
Mouse	Oral	25.0	>	2.7	>	(N.A.)	>	1.0
	Intra-peritoneal	31.0	>	5.2	>	3.0	>	1.0
Rabbit	Oral	9.5	>	.22	>	1.3	>	1.0
	Intra-peritoneal	10.0	>	3.0	>	1.3	>	1.0
Guinea-pig	Oral	(N.A.)		3.9	>	1.6	>	1.0
	Intra-peritoneal	(N.A.)		6.5	>	2.0	>	1.0
Rat	Oral	19	>	6.2	>	1.5	>	1.0
	Intra-peritoneal	40	>	6.1	>	1.1	>	1.0

The nitrophenol derivatives, 2-sec-butyl-4,6-dinitrophenol (dinoseb) and 2,4-dinitro-6-octylphenyl crotonate (binapacryl), have been measured in the blood of animals following oral and dermal applications (Bough et al., 1965). Table 57 presents the results of oral treatment of guinea pigs and the levels of free and total dinoseb in the blood.

Table 57. Blood Levels in Guinea Pigs After Oral Doses of Binapacryl and Dinoseb (Bough et al., 1965)

Parameter		Binapacryl (400 mg/kg)		Dinoseb (40 mg/kg)	
		Group 1	Group 2	Group 3	Group 4
Number of animals		11	4	8	2
Deaths in 7 hours		7	3	8	2
Time of survival (hours)[a]		3.0 onward	5.2 onward	1.7 - 3.0	2.0 and 2.9
Blood concentration of dinoseb (mg/100 ml)[b]					
Before dosing					
Free dinoseb:	Method 2	0.0 (± 0.0) 11	0.0 (± 0.0) 4	0.0 (± 0.0) 8	0.1 (± 0.0) 2
	Method 1	0.1 (± 0.1) 5	0.1 (± 0.0) 4	---	0.1 (± 0.0) 2
Total dinoseb:	Method 1	0.0 (± 0.0) 5	0.0 (± 0.0) 4	---	0.0 (± 0.0) 2
At death					
Free dinoseb:	Method 2	7.6 (± 0.4) 7	8.2 (± 0.8) 3	8.6 (± 0.6) 8	7.8 (± 0.5) 2
	Method 1	7.7 (± 0.3) 5[c]	7.8 (± 0.4) 3	---	8.0 (± 0.5) 2
Total dinoseb:	Method 1	7.6 (± 0.3) 5	7.7 (± 0.4) 3	---	8.4 (± 0.3) 2

a. This applies only to those animals which died within 7 hours.
b. For blood concentrations the values given are means for groups of animals followed by the standard error in parentheses and the number of animals.
c. Estimations made only for five of the seven animals which died.

These results tend to indicate that binapacryl is converted to dinoseb in the body. Both free and total dinoseb levels are about the same at death in all treatment groups, and no evidence of binapacryl was found in the blood of guinea pigs even at large doses.

The outcome of dermal absorption studies presents a different picture, however, as indicated in Table 58.

Table 58. Blood Levels in Rabbits After Dermal Absorption on Binapacryl and Dinoseb (Bough et al., 1965)

	Binaparcryl		dinoseb		
Parameter	750 mg/kg, Group 1	750 mg/kg, Group 2	10 mg/kg, Group 3	20 mg/kg, Group 4	40 mg/kg, Group 5
Number of animals	4	4	4	4	4
Deaths	0	0	0	4	4
Time of survival (hours)	>24	>24	>24	3.5- 5.5	2.5-3
Blood concentration of free dinoseb (mg/100 ml)[a]:					
Before dosing					
Free dinoseb:					
Method 2	0.0(±0.0)4	0.0(±0.0)4	0.0(±0.0)4	0.0(±0.0)4	0.0(±0.0)4
Method 1	--	0.1(±0.0)4	--	--	--
Total dinoseb: Method 1	--	0.0(±0.0)4	--	--	--
1 hour					
Free dinoseb: Method 2	0.0(±0.0)4	--	--	--	--
2 hours					
Free dinoseb: Method 2	0.1(±0.0)4	--	--	--	--
4 hours					
Free dinoseb: Method 2	0.1(±0.0)4	--	--	--	--
8 hours					
Free dinoseb: Method 2	0.2(±0.0)4	--	3.3(±0.3)4	--	--
16 hours					
Free dinoseb: Method 2	--	0.2(±0.1)4	--	--	--
24 hours					
Free dinoseb:					
Method 2	--	0.2(±0.1)4	0.9(±0.2)4	--	--
Method 1	--	0.4(±0.1)4	--	--	--
Total dinoseb: Method 1	--	0.0(±0.0)4		--	--
At death					
Free dinoseb: Method 2	--	--	--	4.5(±0.4)4	5.3(±0.4)4

[a]For blood concentrations, the values given are means for groups of animals followed by the standard error in parentheses and the number of animals.

While rabbits given 20 or 40 mg/kg of dinoseb all died within 5.5 hours, no deaths resulted from applications of binapacryl at concentrations up to 750 mg/kg. Furthermore, blood levels of DNBP in the binapacryl-treated rabbits were not significantly greater than zero, and binapacryl could not be detected in the blood. Therefore, it must be concluded that binapacryl is very poorly absorbed through the skin. This observation is consistent with the results of dermal toxicity studies employing other alkyldinitrophenols with large ring-substituents. For example, 2-cyclohexyl-4,6-dinitrophenol possesses extremely low dermal toxicity (Spencer et al., 1948; see Section III-D) which is probably due to poor absorption through the skin. By comparison, an oral dose of 2-cyclohexyl-4,6-dinitrophenol at 180 mg/kg produced 100% mortality in rats, whereas a topical dose of 1.0 g/kg produced no mortality in the guinea pig. Dinoseb, on the other hand, is rapidly absorbed following dermal application, as can be seen in Figure 32. Even though the time of death varied among the individual rabbits, the blood levels of dinoseb at death are in a very narrow range.

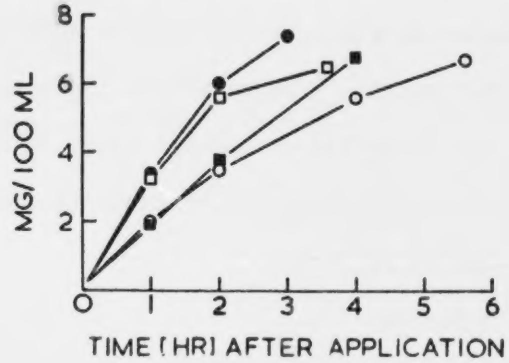

Figure 32. Concentration of DNBP in Blood of Individual Rabbits at Various Times After DNBP (50 mg/kg) Had Been Applied to the Skin (Bough et al., 1965)
(The final point for each animal represents the blood concentration at death.)
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An example of the excretion pattern of a sterically-hindered nitrophenol is provided by a study on the absorption and metabolism of 2,6-di-tert-butyl-4-nitrophenol (BNP) in the rat (Holder et al., 1971). The reported results indicated that BNP is slowly absorbed from the gut following oral administration of ^{14}C-BNP. An average of 28.1 ± 9.8 percent of the dose was excreted as unchanged BNP in the feces. Maximum fecal radioactivity excretion occurred 48 hours after dosing and had ceased after 72 hours. An oral dose of 1.0 mg ^{14}C-BNP in the rat led to the recovery of 33 and 20 per cent of the radioactivity in the urine and feces, respectively, after five days. Pretreatment of the rats with neomycin to kill gut microflora, however, changed these percentages to 23 and 34, respectively. These data would indicate that the gastrointestinal bacteria facilitate the absorption of BNP, as evidenced by the increased fecal radioactivity excretion after antibiotic pretreatment. This conclusion is supported by results obtained from parenteral administration of ^{14}C-BNP to rats. Comparison of the oral and parenteral studies indicated that the excretion of radioactivity in the urine of rats is higher after intraperitoneal injection and suggests that direct absorption of unchanged BNP from the gut is difficult.

b. Nitrobenzene

The absorption of nitrobenzene vapor through the lungs was measured in humans during studies conducted by Salmowa et al. (1963). Seven men were exposed to nitrobenzene in air at concentrations ranging from 5 to 30 μg/ℓ for periods up to six hours. The results indicated that nitrobenzene had been absorbed by the lungs in amounts ranging from 8.4 to 67.6 mg. Figure 33 depicts the time-course of retention of nitrobenzene in the lungs, which averaged 80%, and varied from 87% in the first hour to 73% in the sixth hour.

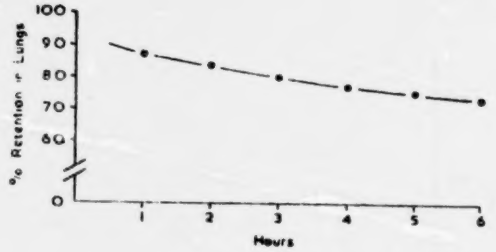


Figure 33. The Percentage Retention of Nitrobenzene Vapor in the Lungs in the Course of a Six-Hour Exposure (Mean Values)
(From Salmowa _et_ _al_., 1963)
(Reprinted from the _British_ _Journal_ _of_ _Industrial_ _Medicine_ with permission from the British Medical Association.)

The kinetics of nitrobenzene elimination were investigated in this study by relating the amount and rate of urinary excretion of _p_-nitrophenol, one of the metabolites of nitrobenzene, to the amount of nitrobenzene absorbed. These relationships are presented in Figures 34 and 35.

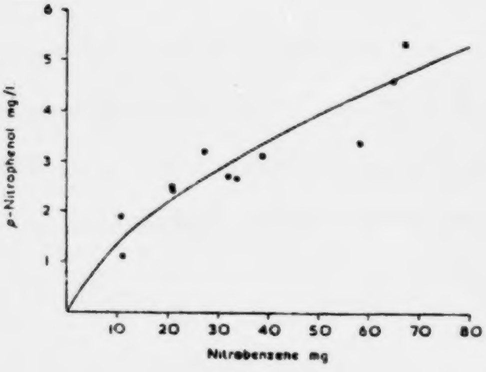


Figure 34. The Concentration of _p_-Nitrophenol in Urine Collected Two to Three Hours After the End of Exposure, as a Function of the Absorbed Dose of Nitrobenzene
(From Salmowa _et_ _al_., 1963)
(Reprinted from the _British_ _Journal_ _of_ _Industrial_ _Medicine_ with permission from the British Medical Association.)

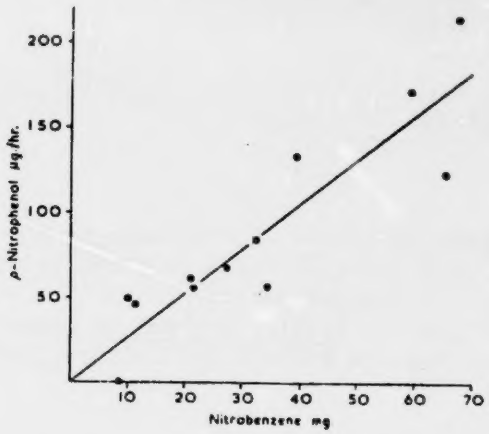


Figure 35. The Excretion Rate of p-Nitrophenol in the Urine Collected During the First Two to Three Hours After the End of Exposure, as a Function of the Absorbed Dose of Nitrobenzene (From Salmowa *et al.*, 1963) (Reprinted from the *British Journal of Industrial Medicine* with permission from the British Medical Association.)

The determination of urinary p-nitrophenol was suggested as a semiquantitative test for nitrobenzene exposure in occupational situations.

A search of the published literature has failed to provide further quantitative data on the absorption or elimination of nitrobenzene derivatives by various routes of administration.

2. Transport and Distribution

Transport and distribution of nitroaromatic chemicals in the body have not been studied extensively. Data from case histories of occupational poisonings have demonstrated that localization of nitrobenzene derivatives in lipids can commonly occur (see Section III-C). It is well known that highly lipid-soluble compounds such as dinitrobenzene and organochlorine pesticides are often localized in adipose tissue due to partition between intracellular lipids and body water.

One can predict that distribution and localization of nitroaromatics in the brain may commonly occur. Exposure to these chemicals often produces pronounced CNS effects, and the uncoupling of oxidative phosphorylation can be demonstrated in brain cell mitochondria of poisoned mice (Ilivicky and Casida, 1969; see Section III-D). It has been pointed out by Schanker (1964) that rapid penetration of drugs into the central nervous system is best accomplished by a substance with a low degree of ionization at blood pH and a high degree of lipid solubility for the undissociated form. These compounds will cross the blood-brain and blood-cerebrospinal fluid barriers at a rate which is related to the lipid/water partition coefficient of the undissociated molecules. On the basis of this information, the passage of many nitroaromatic compounds into the brain can be predicted. These compounds would primarily include those of the nitrobenzene series and their halogen-substituted derivatives. Increasing substitution with nitro, carboxyl, sulfonyl and amino groups would tend to hinder penetration.

Evidence has been presented which demonstrates that *p*-nitroaniline binds to hemoglobin and localizes in the erythrocytes. Schanker *et al.* (1961) measured the binding of *p*-nitroaniline to red cells after incubation with the chemical, followed by rupture of the cells, dilution, and ultrafiltration of the resulting suspensions. These results and the results from incubating p-nitroaniline with varying concentrations of hemoglobin are presented in Figure 36. The binding for undiluted cells was extrapolated to be 77% and agreed closely with theoretical calculations. The extent of binding of p-nitroaniline to hemoglobin was extrapolated from the data to be 72% and, therefore, would account for nearly all of the observed binding of p-nitroaniline to red cells.

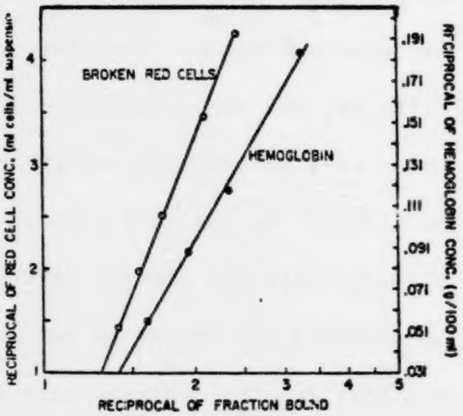


Figure 36. Binding of p-Nitroaniline to Suspensions of Broken Red Cells and to Hemoglobin (Schanker et al., 1961)
(Reprinted from the Journal of Pharmacology (1961), with permission from the Williams & Wilkins Co.)

Numerous studies on the hematologic effects of exposure to nitroaromatic compounds (see Section III-B-4) would tend to indicate that many nitroaromatic substances are capable of entering the red blood cell and combining directly with hemoglobin. For example, acute poisoning by nitrobenzene will typically produce a chocolate-brown discoloration of the blood and a sharp decrease in hemoglobin content as a result of severe hemolysis.

Nakagawa and his associates (1971) examined the distribution of 2,4-dinitrophenyl groups in guinea pig skin after the topical application of 2,4-dinitrochlorobenzene (DNCB). They found that DNCB penetrated through the epidermis into the dermis and combined with skin components within a few minutes after application to the intact skin surface. After 24 hours, only about five percent of the applied DNCB remained in the skin, while the rest had been removed via regional lymphatics and veins to other non-cutaneous tissues or excreted in the urine. Immunofluorescent techniques were used to observe the presence of DNCB in the cytoplasm of epidermal cells, and thereby suggested a possible selective association with cytoplasmic components. This cytoplasmic complex has been postulated to participate in the development of allergic contact sensitivity reactions which commonly occur upon exposure to DNCB or 2,4-dinitrofluorobenzene (Parker and Turk, 1970) (see Section III-B-4-b).

Tissue distribution of DNOC (4,6-dinitro-*o*-cresol) in the rat following subcutaneous injection has been investigated by Parker *et al*. (1951). Their results showed that a single dose of 10 mg/kg DNOC produced very high levels in the serum (100 μg/g at 30 minutes) but no particular accumulation in other tissues. Large amounts of DNOC were detected in the lungs and heart but were postulated to be due to the high blood-content of those organs. The authors calculated that within 30 minutes of the injection, 83% of the DNOC which could be accounted for was present in the blood. After six hours, 0.37 mg of the 1.5 mg dose of DNOC could be accounted for, of which 72% was in the blood.

An examination of the DNOC content of the liver and kidneys was made in rats receiving either a single injection or a series of 40 daily injections of the compound. The results, presented in Table 59, clearly indicated that DNOC had not accumulated in the tissues, nor had the apparent rate of DNOC metabolism apparently been changed by repeated treatment.

Table 59. Comparison of DNOC Concentration in Serum, Kidney, and Liver of Rats After Single and 40 Successive Daily Injections Each of 20 mg DNOC per kg (Parker _et al._, 1951)

Treatment	No. of Rats	Concentration of DNOC 24 Hours after Last Injection (μg/g wet weight)		
		Liver	Kidney	Serum
a. One injection	19	8 ± 0.7μg*	7 ± 0.2μg	45 ± 1.6μg
b. 40 daily injections	9	7 ± 0.3μg	7 ± 0.3μg	38 ± 1.0μg

* Results expressed as means and standard errors.

3. Metabolism and Excretion

a. Nitrobenzene Derivatives

A number of studies have been conducted in an attempt to identify quantitatively the transformation products and to delineate the pathways involved in the mammalian metabolism of nitrobenzene and related nitrobenzene derivatives. Using nitrobenzene randomly labelled with ^{14}C, Parke (1956) accounted for some 85-90% of a single dose of nitrobenzene administered orally to rabbits. Approximately 70% of the dose was eliminated from the body in the expired air, urine, and feces during 4-5 days after dosing. The remainder of the nitrobenzene was retained in the body of the rabbit and slowly excreted in the urine. The metabolic fate of a single oral dose of ^{14}C-nitrobenzene in the rabbit is shown in Table 60.

Table 60. Metabolic Fate of a Single Oral Dose of ^{14}C-Nitrobenzene in the Rabbit During 4-5 Days After Dosing (Parke, 1956)

Metabolite	Percentage of Dose (Average)			
Respiratory CO_2	1	2 in expired air		60 total
Nitrobenzene	0.6*			
Aniline	0.4†		58 in urine	
o-Nitrophenol	0.1			
m-Nitrophenol	9			
p-Nitrophenol	9			
o-Aminophenol	3			
m-Aminophenol	4			
p-Aminophenol	31			
4-Nitrocatechol	0.7			
Nitroquinol	0.1			
p-Nitrophenylmercapturic acid	0.3			
(total urinary radioactivity)	(58)			
Metabolized nitrobenzene in feces	9^Δ			
Metabolized nitrobenzene in tissues	15-20			
Total nitrobenzene accounted for	85-90%			

* 0.5% in the expired air and < 0.1% in the urine.

† 0.3% in the urine and < 0.1% in the expired air.

Δ 6% of the dose was present in the feces as *p*-aminophenol

Of the 70% of the dose eliminated from the body, about 58% was excreted in the urine. The major urinary metabolite, *p*-aminophenol, accounted for 31% of the dose.

These quantitative findings are in good agreement with those obtained by Robinson *et al.* (1951 a) in a previous study on the metabolism of nitrobenzene in the rabbit using the unlabelled chemical. From their results, the authors have proposed the scheme shown in Figure 37, depicting possible pathways of nitrobenzene metabolism.

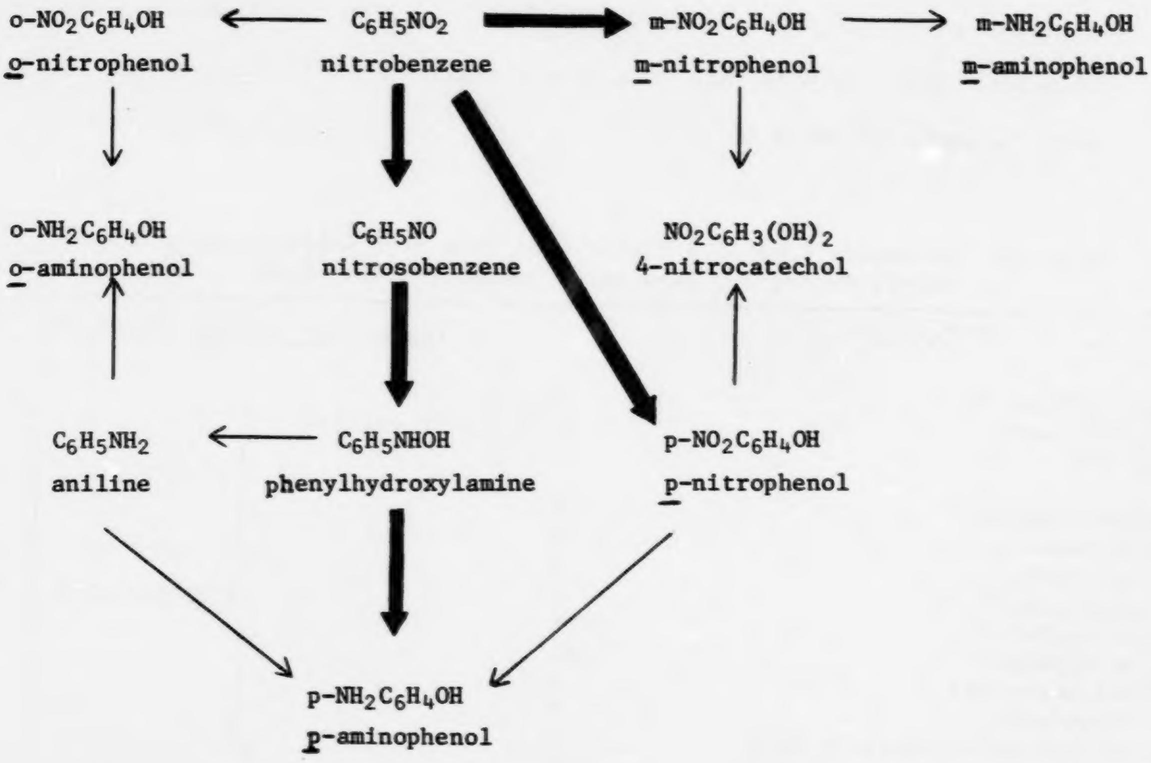


Figure 37. Suggested Pathways in the Metabolism of Nitrobenzene (Robinson *et al.*, 1951 a) (Major metabolic paths are shown by heavy arrows.)

Analyses of urine samples revealed that the *m*- and *p*-nitrophenols were produced in roughly equal quantities, wheras the amount of *p*-aminophenol produced was very much greater than the *m*-aminophenol. From this, it was suggested that the major portion of the *p*-aminophenol was not derived from *p*-nitrophenol. Since *p*-aminophenol occurred in much greater amounts than *o*-aminophenol, it was postulated that *p*-aminophenol was not primarily derived from aniline. Therefore, since a hydroxylamine has been established as an intermediate in the biological reduction of nitro compounds (Channon *et al.*, 1944), it was concluded that the major portion of the *p*-aminophenol may have arisen from nitrobenzene via phenylhydroxylamine.

The metabolism of *m*-dinitro[^{14}C]benzene in the rabbit at oral doses of 50-100 mg/kg body weight has been studied (Parke, 1961). In two days, some 65-93% of the dose was excreted in the urine and 1-5% in the feces. The remainder of the radioactivity was presumed to be retained in the tissues and slowly excreted in the urine. The metabolites of *m*-dinitro[^{14}C]benzene excreted in the urine are listed in Table 61.

The reduction products, *m*-nitroaniline and *m*-phenylenediamine, were among the major metabolites, together accounting for 35% of the dose. Some 1% of the dose was excreted as *m*-nitrophenylhydroxylamine and artifacts derived therefrom (e.g., 3,3'-dinitroazoxybenzene and *m*-nitrosonitrobenzene). Traces of unchanged *m*-dinitrobenzene were excreted (0.7%).

The major phenolic metabolite of *m*-dinitrobenzene was 2,4-diaminophenol which accounted for 31% of the dose. 2-Amino-4-nitrophenol, also a principal metabolite, was excreted as 14% of the dose. 2,4-Dinitrophenol was found to be present in trace amounts (0.1%). Approximately 30% of the dose is excreted as glucuronide conjugates and 6% as ethereal sulphates after administering non-radioactive *m*-dinitrobenzene.

Table 61. Metabolites of m-Dinitro[^{14}C]benzene Excreted in Urine by Rabbits (from Parke, 1961) (m-Dinitrobenzene was administered orally as aqueous suspensions. Urines were collected for two days.)

Expt. no.	1*	2	3	4	5	6	7	
Dose of m-dinitrobenzene (mg/kg)	100	70	70	60	60	50	50	
Dose of ^{14}C (μc/animal) ...	5	4	5	4	4	2	3	Average
	Percentage of dose							
m-Dinitrobenzene	<0.1	0.3	---	2.4	---	0.4	0.7	0.7
m-Nitrophenylhydroxylamine	---	---	---	---	0.7	1.1	0.75	0.8
m-Nitrosonitrobenzene	---	---	---	0.5	0.2	0.1	0.25	0.25
3,3'-Dinitroazoxybenzene	---	---	---	0.3	0.2	0.3	---	0.3
m-Nitroaniline (total)	28	35	10	18	---	---	---	35
m-Phenylenediamine (total)	<0.2	<0.2	23	25	---	---	---	
2,4-Dinitrophenol (total)	0.1	0.1	<0.1	---	---	---	---	0.1
2-Amino-4-nitrophenol (total)	12.5	15	12	16	---	---	---	14
4-Amino-2-nitrophenol (total)	1.4	1.6	2.1	2.6	---	1.9 (0.6)†	2.4 (1.0)†	2.0 (0.8)†
2,4-Diaminophenol (total)	19	37	38	28	---	---	---	31
Total metabolites	61	89	85	93	---	---	---	83
Total radioactivity in urine	65	89	82	93	71	89	75	81
Total radioactivity in feces	---	0.3	1.0	---	5.2	---	---	---

*Animal died on 3rd day.

† Estimated after enzymic hydrolysis.

One possible sequence in the metabolism of m-dinitrobenzene has been presented by Williams (1959) and is shown in Figure 38.

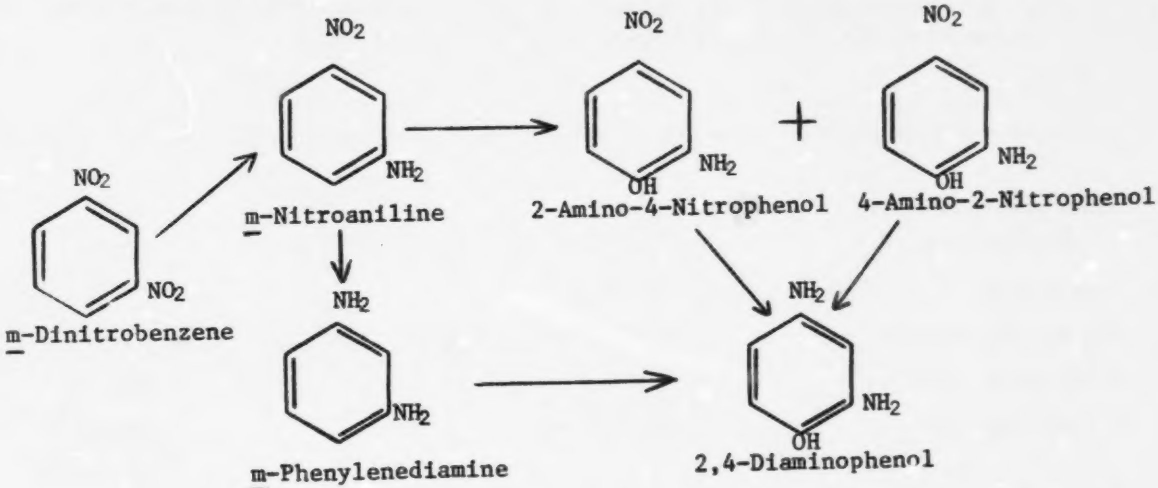


Figure 38. Metabolism of m-Dinitrobenzene (from Williams, 1959)

A study of the metabolic fate of o-, m-, and p-chloronitrobenzene in the rabbit has shown that reduction and hydroxylation are the major metabolic processes acting on these compounds (Bray *et al.*, 1956). The main urinary excretion products are phenols conjugated with glucuronic and sulphuric acids. Chloroanilines, chloronitrophenols, and aminochlorophenols were found to be metabolites of all the chloronitrobenzenes. A summary of the results obtained from quantitative analysis of urine from rabbits dosed with 0.1 g/kg o-chloronitrobenzene, and 0.2 g/kg m- and p-chloronitrobenzenes is shown in Table 62.

Table 62. Excretion of Metabolites of *o*, *m*-, and *p*-Chloronitrobenzenes by the Rabbit (from Bray *et al.*, 1956)

- Results are expressed as percentages of the dose, given as means with ranges in parentheses; superscript figures indicate the number of experiments. Consecutive urine samples were analysed until excretion of metabolites ceased. The unabsorbed material found in feces was completely reduced to the chloroaniline except in the case of the *para*-isomer, when it consisted of approximately 1 part of *p*-chloronitrobenzene and 2 parts of *p*-chloroaniline. The values for chloroanalines were obtained from steam distillates of the pooled urines of six rabbits.

Chloronitrobenzene administered	ortho-	meta-	para-
Unabsorbed	0.3 (0.1,0.5)^2	0.6 (0.5,0.7)^2	2.8 (2.4,3.2)^2
Ether glucuronide	42 (26-56)^3	33 (17-58)^9	19 (9-27)^4
Ethereal sulphate	24 (18-31)^3	18 (4-30)^6	21 (15-37)^4
Mercapturic acid	7 (0-18)^3	1 (0-1)^5	7 (2-11)^6*
			3 (0-19)^9**
Chloroaniline, free	9	11	9
Chloroaniline, conjugated	0	0	4
Total accounted for	82	64	63

* Colorimetric method. This method was more sensitive than the Stekol method and indicated that small amounts (1% of dose) were excreted on the fourth day after dosage.

** Values by modified Stekol method.

- Small amounts of unconjugated phenolic metabolites were not included in the values given for the total percentage of the dose accounted for.

Possible pathways leading to the formation of the phenolic metabolites of the chloronitrobenzenes have been suggested by Bray *et al*. (1956) (Figure 39). As shown in Figure 39, reduction could precede or follow hydroxylation.

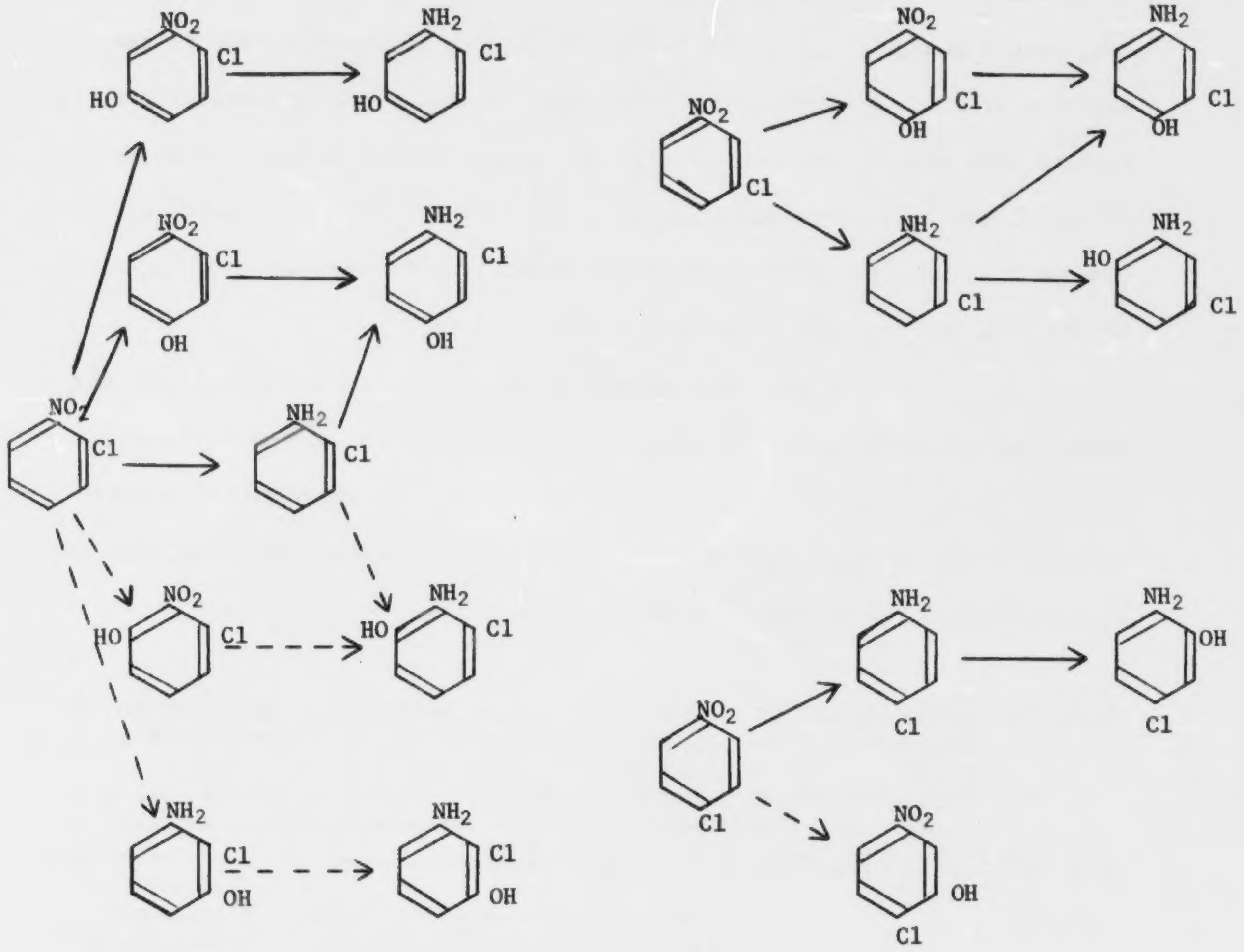


Figure 39. Phenolic Metabolites Excreted (Free or Conjugated) in the Urine by the Rabbit After Dosage with *o*-, *m*- and *p*-Chloronitrobenzene and *o*-, *m*- and *p*-Chloroaniline (from Bray *et al*., 1956)
(Broken arrows point to metabolites excreted only in very small amounts. Although only a small amount of 4-chloro-3-nitrophenol was excreted, it is likely that a much greater amount was formed and reduced to 3-amino-4-chlorophenol before it was excreted.)

A study of the metabolism of orally administered 2,3,5,6- and 2,3,4,5-tetrachloronitrobenzene in the rabbit demonstrated that these compounds were not readily adsorbed and that some reduction of the nitro group occurred in the intestine (Bray et al., 1953). Excretion of 2,3,5,6-tetrachloronitrobenzene in the feces was complete within three days after dosing and, over a dosage range of 0.1-3.0 g, 59-78% was unabsorbed. Due to the toxicity of 2,3,4,5-tetrachloronitrobenzene, dosage levels administered to the rabbits were usually not greater than 0.3 g/kg. During 48 hours after dosage, 27-36% of the 2,3,4,5-compound was excreted in the feces as a mixture of unchanged 2,3,4,5-tetrachloronitrobenzene and tetrachloroaniline. Of this 27-36%, 20% was reported to be in the reduced form.

Urinary metabolites of the tetrachloronitrobenzenes were identified as tetrachloroaniline, tetrachloroaminophenol and glucuronide, ethereal sulfate, and mercapturic acid conjugates. A comparison of quantitative results obtained with 2,3,5,6- and 2,3,4,5-tetrachloronitrobenzene and 2,3,5,6-tetrachloroaniline is shown in Table 63.

Table 63. Excretion of Metabolites of 2,3,5,6- and 2,3,4,5-Tetrachloronitrobenzenes and of 2,3,5,6-Tetrachloroaniline by the Rabbit (Bray et al., 1953)
(Results are expressed as the average percentage of the absorbed dose. Absorbed dose = actual dose administered less material found in feces after 48 hours.)

	2,3,5,6-Tetrachloronitrobenzene	2,3,5,6-Tetrachloroaniline	2,3,4,5-Tetrachloronitrobenzene
Absorbed dose (g)	0.58	0.82	0.47
Metabolite:			
Tetrachloroaniline	23	18	16
Tetrachloroaminophenol	5	--	--
Glucuronide	31	73	61
Ethereal sulphate	3	12	9
Mercapturic acid	28	0	0
Total accounted for	90	103	86

The fact that the administration of tetrachloroaniline gave no detectable mercapturic acid suggested that the mercapturic acid conjugate was formed only from the nitro compound or an intermediate in its reduction. The following scheme summarizes the experimental results obtained by Bray et al. (1953) using a dose of 1.5 g of 2,3,5,6-tetrachloronitrobenzene:

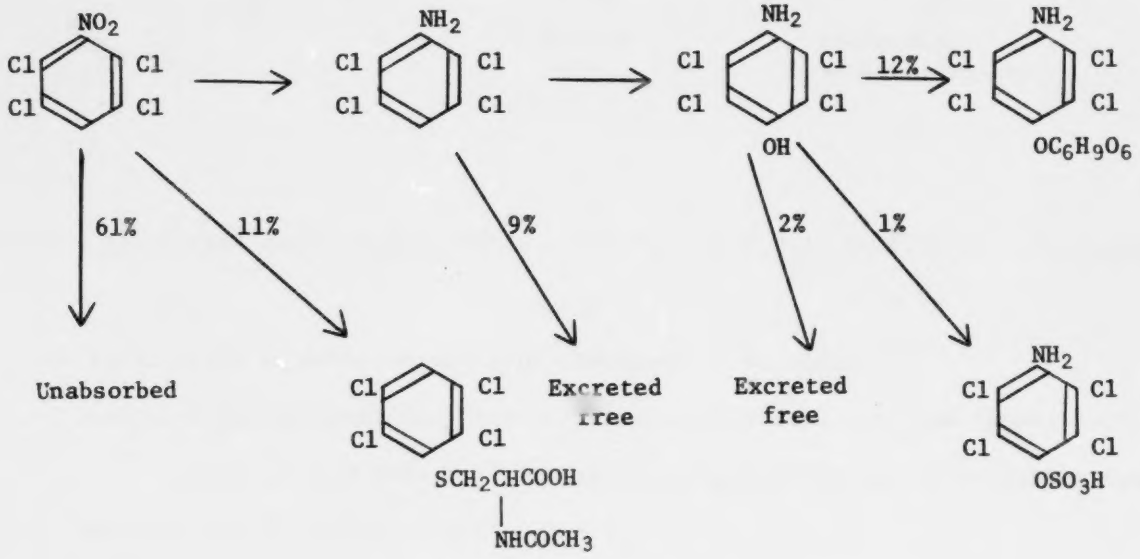


Figure 40. Metabolism of 2,3,5,6-Tetrachloronitrobenzene (Bray et al., 1953)

The metabolism of 2,3,4,5-tetrachloronitrobenzene differed considerably from that of the 2,3,5,6-isomer. A greater amount of the 2,3,4,5-compound was absorbed, the extent of hydroxylation was greater, and no mercapturic acid formation was detected. Using a dose of 0.7 g of 2,3,4,5-tetrachloronitrobenzene, Bray and coworkers (1953) observed the results presented in Figure 41.

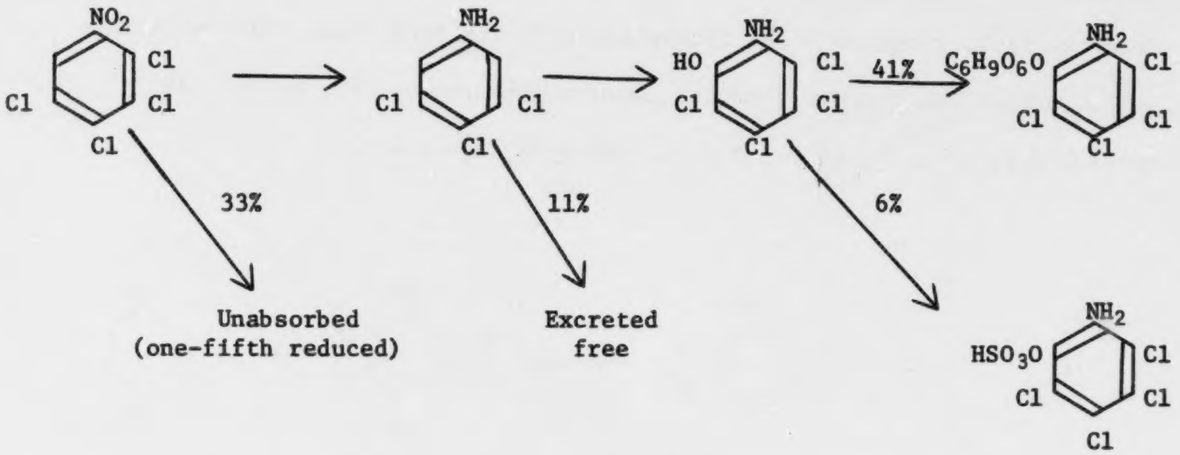


Figure 41. Metabolism of 2,3,4,5-Tetrachloronitrobenzene (from Bray et al., 1953)

Since nitro compounds were not detected as metabolites of either isomer and all identified metabolites were anilines, it was suggested that reduction of the tetrachloronitrobenzenes precedes hydroxylation.

Studies on the metabolism and excretion of nitrobenzene derivatives have also included an investigation of pentachloronitrobenzene (PCNB) (Kuchar et al., 1969). Feeding studies were conducted using beagle dogs and rats which were treated with various levels of PCNB in the diet for up to two years. Table 64 presents the analysis of tissues from three dogs fed PCNB at levels of 5 and 1080 ppm in the diet over a two year period. Chromatographic examination of hexane extracts of the tissues confirmed the formation of four major metabolites: pentachlorobenzene (PCB), hexachlorobenzene (HCB), pentachloroaniline (PCA), and methylpentachlorophenyl sulfide.

As indicated in Table 64, accumulation of PCB and HCB, either as metabolites or chlorinated impurities in the treatment compound, had occurred in the fat. Additional studies in rats were performed whereby PCNB was fed in the diet for seven months at levels of 50 and 500 ppm. Some groups were put on a control diet for two months after the end of PCNB treatment before being sacrificed. An analysis of the fat from these animals is presented in Table 65; the results indicate storage of HCB in the fat which persists after the cessation of treatment.

A further analysis of the urine was made to determine the extent of metabolic conjugation of PCA with glucuronic and sulfuric acid. Hydrolysis of the urine with sulfuric acid resulted in an increase in PCA content, and thereby confirmed the role of the conjugation process (Table 66).

b. Nitrotoluene Derivatives

The oxidation of the mononitrotoluenes was studied at an early date (1874) by Jaffe in an investigation of the fate of o- and p-nitrotoluene in dogs. The methyl group of o-nitrotoluene was found to be oxidized to yield o-nitrobenzyl alcohol and o-nitrobenzoic acid. The alcohol, excreted as a glucuronide, accounted for 25% of the dose; the o-nitrobenzoic acid, 10%. The metabolism of p-nitrotoluene resulted in the formation of p-nitrobenzoic acid and p-nitrohippuric acid (Williams, 1959).

Gillette (1959) studied an enzyme system in rabbit liver that catalyzed the oxidation of p-nitrotoluene to p-nitrobenzoic acid (PNBA). He found that neither the soluble nor the microsomal fractions of rabbit liver homogenates alone could transform p-nitrotoluene to PNBA. It was shown that p-nitrotoluene was first metabolized to p-nitrobenzyl alcohol by a TPNH-dependent

Table 64. PCNB Studies on Twenty-Four Month Male Beagle Dog Tissues (from Kuchar et al., 1969)

	PCNB	Data in ppm PCB	HCB	PCA	MPS[a]
Muscle					
5 ppm PCNB	ND	< 0.003	0.016	ND	ND
1080 ppm PCNB	ND	0.234	7.28	ND	0.227
Kidney					
5 ppm PCNB	ND	0.012	0.035	ND	ND
1080 ppm PCNB	ND	0.214	6.41	ND	1.08
Fat					
5 ppm PCNB	ND	0.093	0.452	0.010	0.030
1080 ppm PCNB	ND	5.15	194	0.643	2.50
Liver					
5 ppm PCNB	ND	0.007	0.039	0.057	0.039
1080 ppm PCNB	ND	0.387	5.92	0.037	0.322
Urine[b]					
5 ppm PCNB	ND	ND	ND	<0.002	<0.001
1080 ppm PCNB	<0.004	ND	<0.001	0.092	<0.001
Feces[b]					
5 ppm PCNB	0.059	0.007	0.009	0.188	0.134
1080 ppm PCNB	14.1	0.422	1.37	16.7	3.64

a. Methyl pentachlorophenyl sulfide.
b. Twenty-four hour samples before scarifice.
ND - None Detected

Table 65. PCNB Studies on Rat Fat (from Kuchar *et al.*, 1969) (Data in ppm)

PCNB Level in Food ppm	PCNB	PCB	HCB	PCA	MPS[a]
	Male Rats Fed PCNB Seven Months, Sacrificed, and Fat Analyzed				
50	ND	0.019	10.8	0.019	0.46
500	ND	0.304	117	1.11	4.74
	Male Rats Fed PCNB Seven Months, Then on Control Diet for Two Months, Fat Analyzed				
50	ND	ND	3.67	ND	ND
500	ND	ND	22.3	ND	ND

a. Methyl pentachlorophenyl sulfide.
ND - None Detected.

Table 66. Acid Hydrolysis vs. Direct Solvent Extraction (from Kuchar *et al.*, 1969) (Data in ppm)

	PCA	
Urine	H+	Direct Solvent
Male Dog 56	0.097	<0.005
(1080 ppm PCNB)	0.079	<0.004
Male Dog 59	1.14	0.354
(1080 ppm PCNB)	2.92	0.164
Liver		
Male Dog 56	0.111	0.028
(1080 ppm PCNB)	0.092	0.034
Male Dog 59	0.195	0.044
(1080 ppm PCNB)	0.220	

enzyme system located in the microsomal fraction of liver. A second enzyme, localized in the soluble fraction of liver, metabolized p-nitrobenzyl alcohol to PNBA by a two-step DPN-dependent process. First, p-nitrobenzyl alcohol was oxidized to p-nitrobenzaldehyde by an alcohol dehydrogenase; then followed by the oxidation of p-nitrobenzaldehyde to PNBA by an aldehyde dehydrogenase. This scheme would appear as follows:

$$\text{p-nitrotoluene} \xrightarrow[\text{microsomes}]{\text{TPNH} + O_2} \text{p-nitrobenzyl alcohol}$$

$$\text{p-nitrobenzyl alcohol} \xrightarrow[\substack{\text{alcohol}\\ \text{dehydrogenase}}]{\text{DPN}} \text{p-nitrobenzaldehyde}$$

$$\text{p-nitrobenzaldehyde} \xrightarrow[\substack{\text{aldehyde}\\ \text{dehydrogenase}}]{\text{DPN}} \text{PNBA}$$

The oxidation of p-nitrotoluene by several insect species has revealed the formation of p-nitrobenzoic acid as the only metabolite detected (Chakraborty and Smith, 1964). Since alkyl side chains are structural features of several insecticides, the study of the metabolism of such alkyl groups is of interest. As Chakraborty and Smith have suggested, different rates of oxidation of insecticides having aliphatic side chains could be a factor in differing rates of detoxication.

The metabolism of 2,4,6-trinitrotoluene (α-TNT) was studied by Channon et al. (1944) in an attempt to learn the fate of trinitrotoluene in the body. The isolation of 2,6-dinitro-4-hydroxylaminotoluene, 2,6-dinitro-4-aminotoluene, and 2,4-dinitro-6-aminotoluene from the urine of rabbits receiving 2,4,6-trinitrotoluene demonstrated the existence of the reduction mechanism:

$$CH_3C_6H_2(NO_2)_3 \longrightarrow CH_3C_6H_2(NO_2)_2NHOH \longrightarrow CH_3C_6H_2(NO_2)_2NH_2$$

The 2,2',6,6'-tetranitro-4,4'-azoxytoluene, which had been previously obtained from the urine of rabbits (Dale, 1921) and from workers in TNT factories (Moore, 1917; Webster, 1921), was shown not to be a metabolic product. The azoxytoluene appears to be an artifact present in freshly voided urine. Thus, there was no evidence for the in vivo formation of the azoxy compound.

About 30% of the trinitrotoluene administered was excreted as aromatic amino compounds and 47% in combination with glucuronic acid.

In an investigation of the chemical nature of the red pigment present in urine after exposure to TNT, the authors administered possible intermediate metabolic substances to rats and observed the color of the urine excreted. Of the compounds used, only α-TNT and 2,4,6-trinitrobenzyl alcohol caused the urine to be red. Thus, the red pigment in trinitrotoluene urine may be due to the presence of 2,4,6-trinitrobenzyl alcohol or one of its derivatives. This finding indicates the possible existence of the oxidation mechanism:

$$CH_3C_6H_2(NO_2)_3 \longrightarrow HOH_2CC_6H_2(NO_2)_3$$

Doses of up to 150 mg/kg of α-TNT were eliminated within 24 hours; elimination of larger doses required up to 48 hours. The excretion of unchanged α-TNT was not observed.

c. Nitroaniline Derivatives

The metabolism of several derivatives of 4-nitroaniline in the rat has been studied by Mate et al. (1967). Treatment of rats with [^{14}C]2,6-dichloro-4-nitroaniline by oral and intraperitoneal routes produced an excretion pattern for ^{14}C as outlined in Table 67.

Isolation of radioactive metabolites by reverse isotope dilution of the 24-hour urine revealed that 70.4 ± 3.9 percent of the urinary radioactivity was in the form of 4-amino-3,5-dichlorophenol. An additional 2.4 ± 0.3 percent of the activity was present as 4-amino-2,6-dichloroaniline. There was no evidence of any parent 2,6-dichloro-4-nitroaniline being present in the urine. Another *in vivo* study using ^{14}C-labelled 2,6-dibromo-4-nitroaniline produced a similar pattern of metabolic reduction. After intraperitoneal injection of 5 mg of starting material, 81 ± 3 percent of the dose was excreted in the 24-hour urine, of which 80 percent was 4-amino-3,5-dibromophenol. None of the parent compound was present.

When rats were treated with unsubstituted [^{14}C]4-nitroaniline, about 80 percent of the dose was excreted in the 24-hour urine regardless of the route of administration (Table 68).

Analysis of the acid-hydrolyzed urine by reverse isotope dilution demonstrated that 14.1 ± 2.0 percent of the activity was 4-nitroaniline, 26.1 ± 6.8 percent was 4-phenylenediamine, and 43.1 ± 2.5 percent was 2-amino-5-nitrophenol. The unhydrolyzed urine contained only two percent 4-nitroaniline in the unconjugated form.

The mechanism of formation of aminophenols from halogenated nitroanilines was postulated to begin with N-hydroxylation, as illustrated in

Table 67. Excretion of ^{14}C by Rats Dosed with $[^{14}C]$2,6-Dichloro-4-nitroaniline (from Mate *et al.*, 1967)

		Percentage of dose excreted in					
		Urine			Feces	Bile	
Route of Administration	Dose (mg/rat)	24 hr	48 hr	72 hr	72 hr	6 hr	12 hr
Intraperitoneal	10	69.7 ± 4.5	11.9 ± 5.2	1.0 ± 0.5	1.5 ± 0.5		
Oral	10	77.1 ± 3.8	13.75 ± 6.1	0.5 ± 0.3	1.0 ± 0.4		
Intraperitoneal	5					1.0 ± 0.9	
Oral	5						5.3 ± 0.8

Table 68. Excretion of ^{14}C by Rats Dosed with [^{14}C]4-Nitroaniline (from Mate *et al.*, 1967)

Route of administration	Dose (mg/rat)	Percentage of dose excreted in			
		Urine			Feces
		24 hr	48 hr	72 hr	48 hr
Intraperitoneal	5	76.5 ± 1.4	2.8 ± 1.4	1.6 ± 0.6	0.4 ± 0.2
Oral	5	83.0 ± 1.7	1.7 ± 0.4	1.0 ± 0.8	0.6 ± 0.3

Results are expressed as the mean ± SE. A group of six rats was used in each experiment.

Figure 42. This is followed by nucleophilic attack by water, and then proton rearrangement leading to quinonimine formation which is then reduced to the aminophenol.

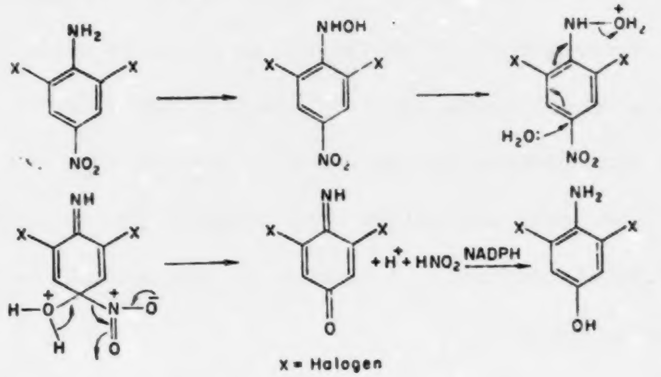


Figure 42. Proposed Mechanism for the Displacement of Nitro by Hydroxyl in the Metabolism of 2,6-Dihalo-4-Nitroanilines (Mate et al., 1967) (Reprinted with permission from C. Mate, A.J. Ryan, and S.E. Wright, "Metabolism of Some 4-Nitroaniline Derivatives in the Rat" (1976), Food Cosmet. Toxicol., Pergamon Press Ltd.)

The replacement of the nitro group by the hydroxyl group appeared to be limited to the halogenated compounds, however. In the case of 4-nitroaniline, it was suggested that there is no hindrance to nucleophilic attack at the ortho position. Therefore, rearrangement of the hydroxylamine would preferentially lead to 2-amino-5-nitrophenol, as was reported in these experiments.

d. Nitrophenol Derivatives

A study of the metabolism of the *o*-, *m*-, and *p*-nitrophenols in the rabbit has shown that, with doses of 0.2-0.3 g/kg, conjugation *in vivo* with glucuronic and sulfuric acids was almost complete (Robinson *et al*., 1951 b). With all three of the mononitrophenols, the major conjugation product was nitrophenyl glucuronide. Reduction of the nitrophenols occurred to a small extent, the reduction of the *p*-isomer being slightly greater than that of the *m*- and *o*-isomers. That the nitrophenols are oxidized *in vivo* to dihydric nitrophenols has been demonstrated by paper chromatography; however, the oxidation products comprise less than 0.5% of the dose. A summary of the metabolism of the mononitrophenols is shown in Table 69.

Table 69. Summary of the Metabolism of Mononitrophenols (from Robinson *et al*., 1951 b)

	Percentage of Dose Excreted as					
	Nitro Compounds	Amino Compounds		Glucuronides	Ethereal Sulphates	
Nitrophenol	N	A	(N + A)*	G	E	(G + E)*
Ortho	82	3†	85	71	11	82
Meta	74	10	84	78	19	98
Para	87	14	101	65	16	81

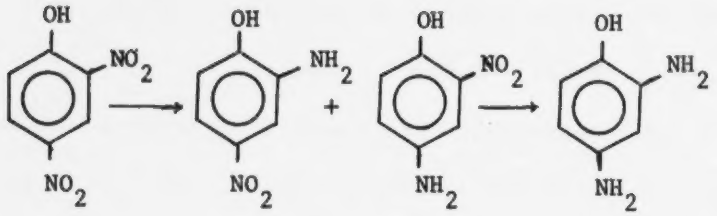
* (N + A) should be roughly equal to (G + E), since the amounts of free phenols excreted were very small.

† This figure is low since only *o*-aminophenol combined with glucuronic acid was estimated. To allow for *o*-aminophenol combined with sulfuric acid, this figure could probably be doubled.

Parker (1952) studied the enzymatic reduction of dinitrophenol by rat liver homogenates in order to ascertain the metabolic products. Of the possible amino derivatives, 4-amino-2-nitrophenol was found to be the major metabolite, comprising 90% of the total metabolites formed, and 2-amino-4-nitrophenol accounted for less than 10% of the amines produced. Definite identification of the second reduction product, 2,4-diaminophenol, was not made. In an investigation of the rate of destruction of dinitrophenol and the rate of formation of amino compounds, loss of amine occurred after three hours, while destruction of dinitrophenol continued. When the two aminonitrophenols were incubated with liver homogenates, the 2-amino-4-nitrophenol was slowly destroyed, while 4-amino-2-nitrophenol was destroyed more rapidly. In an in vivo study of rats poisoned by 2,4-dinitrophenol, the urinary excretion products were unchanged 2,4-dinitrophenol and 2-amino-4-nitrophenol. There was no evidence of 4-amino-2-nitrophenol. Since the 4-amino isomer was destroyed more rapidly in vitro than the 2-amino compound, the authors suggested that 2-amino-4-nitrophenol might be expected to appear as the main reduction product of dinitrophenol in the urine.

Eiseman et al. (1972) examined the in vitro metabolism of ^{14}C-2,4-dinitrophenol by rat liver homogenates. Of the 81±4% of the dinitrophenol metabolized during a 30-minute incubation period, 75±4% was metabolized to 2-amino-4-nitrophenol, 23±2% to 4-amino-2-nitrophenol, and 1% to 2,4-diaminophenol. These amounts differ significantly from Parker's results, in which Parker reported 4-amino-2-nitrophenol to be the major metabolite. Eiseman et al. (1972) stated that a contributing factor in the difference in results could be the lower stability of the 4-amino isomer and its possible oxidation to a non-extractable product.

Although the quantitative aspects of the metabolism of 2,4-dinitrophenol remain to be elucidated, the outline of its metabolic fate as reported by Williams (1959) is as follows:



A recent investigation on the disposition of 4,6-dinitro-2-sec-butylphenol (dinoseb) in female mice was conducted by Gibson and Rao (1973). It had previously been established that dinoseb is teratogenic in mice (Gibson, 1973; see Section III-D-5). When administered by oral and intraperitoneal routes, ^{14}C-dinoseb was excreted in the urine, feces, and bile as outlined in Table 70.

Three hours after administration to pregnant mice, the liver and kidney were found to contain about 50 percent unchanged dinoseb and 50 percent as unidentified metabolites. In the embryo, 85 percent of the radioactivity present three hours after the oral administration of dinoseb was in the

Table 70. Excretion of $[^{14}C]$Dinoseb by Female Mice Following Oral or IP Administration (from Gibson and Rao, 1973)

		Mean cumulative excretion (% of administered radioactivity) in					
		Bile		Urine		Feces	
Time after administration (hr)	Treatment.... Dose (mg/kg).	Oral 32	Ip 17.7	Oral 32	Ip 17.7	Oral 32	Ip 17.7
0.5		0.1 ± 0	0.2 ± 0.1	-	-	-	-
1		0.4 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.4 ± 2.0	-	-
2		0.6 ± 0.1	1.4 ± 0.4	1.9 ± 0.4	3.9 ± 0.1	-	-
4		0.9 ± 0.4	3.9 ± 0.6	3.2 ± 0.4	7.0 ± 0.1	-	-
8		1.4 ± 0.6	9.6 ± 1.4*	6.8 ± 1.4	13.4 ± 1.3	0.5 ± 0	3.3 ± 0.9
16		-	-	14.4 ± 2.0	22.1 ± 2.1	4.3 ± 1.1	11.1 ± 1.1*
32		-	-	23.2 ± 3.5	26.3 ± 1.9	9.7 ± 3.7	28.7 ± 4.8*
64		-	-	26.3 ± 3.3	28.2 ± 2.5	30.4 ± 7.5	40.8 ± 6.5

Values are means for groups of three mice ± SEM. Those marked with asterisks differ significantly from the corresponding value for orally treated animals: $*P < 0.05$.

form of the unchanged compound. After intraperitoneal injection, however, only 57 percent of the radioactivity in the embryo was present as unchanged dinoseb. This difference may explain the apparent lack of teratogenic and embryotoxic effects of dinoseb when it is administered by the oral route, as opposed to its definite toxic effects when given intraperitoneally (Gibson, 1973). Such observations illustrate the importance of conducting studies in toxicologic metabolism prior to making judgements concerning the potential hazards of environmental chemicals.

e. Metabolic Reduction of Nitroaromatic Compounds

A comprehensive investigation of the *in vitro* metabolism of numerous nitroaromatic compounds was conducted by Fouts and Brodie (1957). They described a nitro reductase system present in the liver and kidney of rabbits which can reduce various nitroaromatic compounds to the corresponding amines. The system was localized in both the soluble fraction and the microsomes of the liver cell. This system was completely inactivated in the presence of oxygen. Both TPNH and DPNH could act as hydrogen donors for the system, which also included a flavoprotein whose prosthetic group may be replaced by any of the three flavins, riboflavin-5-phosphate, flavin adenine dinucleotide, or riboflavin. The wide variety of nitroaromatic compounds which could be reduced by these enzymes is illustrated in Table 71. The nitrophenols are notable exceptions to the rapid reducing action of the system described.

The formation of arylhydroxylamines as intermediates in the metabolic reduction of nitro compounds is of great significance in determining their threat to human health. Sternson (1975) has pointed out that the carcinogenicity of nitroaromatic compounds may depend upon their metabolic

Table 71. Reduction of Various Nitro Compounds by Liver Homogenates (from Fouts and Brodie, 1957)

Supernatant fraction (900 x g) equivalent to 1 gm liver, 4 micromoles of substrate, 0.6 micromole of TPN, and 100 micromoles of nicotinamide were incubated for two hours at 37°C with nitrogen as the gas phase.

Substrate	Reduced Product Formed (μmoles)	Relative Optical Density (540 mμ) of Reduced Product
Chloramphenicol	3.07	0.650
_m_-Dinitrobenzene	1.83	1.150
_p_-Nitrotoluene	1.60	0.230
_p_-Nitrobenzoic acid	1.50	1.335
Nitrobenzene	1.15	0.335
_m_-Nitrobenzoic acid	0.61	0.550
2,4-Dinitrophenol	0.10	0.075
_p_-Nitrophenol	0.20	0.150
_m_-Nitrophenol	0.00	0.010
_p_-Nitrobenzyl alcohol	--	0.840
_m_-Nitroacetophenone	--	0.620
_m_-Nitrobenzaldehyde	--	0.530
_p_-Nitrobenzaldehyde	--	0.530

activation to the corresponding hydroxylamine (see Section III-D-6). He studied the reductive metabolism by the action of hepatic nitro reductases of a series of nitroaromatic compounds in rabbit liver microsomal suspensions. The detection of hydroxylamine intermediates formed by metabolic reduction in biological systems had previously been very difficult due to their high reactivity and lability. A sensitive electrochemical detection method was employed, however, based on the anodic oxidation of hydroxylamines at carbon paste electrodes. By using this technique, hydroxylamines were demonstrated in liver microsome incubations containing either 1-nitronaphthalene, nitrobenzene, nitrofluorene, or p-nitrocresol. These reactions were carried out under anaerobic conditions because the presence of oxygen destroyed nitro reductase activity, which indicated that the process was mediated by cytochrome P-450.

In an investigation of the enzymatic reduction of 2-nitronaphthalene and similar nitroaromatic compounds by rat liver in vitro, 2-naphthylamine was slowly produced as 2-nitronaphthalene disappeared (Poirier and Weisburger, 1974). Similarly, the reduction of 1-nitronaphthalene led to the formation of the corresponding arylamine. In both cases, there was no evidence of hydroxylamine accumulation. Previous studies on the in vitro enzymatic reduction of p-nitrobenzoic acid (Kato et al., 1969), nitrobenzene (Uehleke, 1963), and 4-nitrobiphenyl (Uehleke and Nestel, 1967) reported the formation of hydroxylamine intermediates. Poirier and Weisburger (1974) attributed their lack of detection of arylhydroxylamines to a low activity of nitroreductase and the fact that the arylhydroxylamines, when formed, are rapidly reduced to amines by liver extracts.

f. Metabolism by Gastro-Intestinal Microorganisms

The reduction of nitro groups by intestinal microorganisms has been known for some time (Glazko *et al.*, 1949; Zachariah and Juchau, 1974) and can often determine the form in which a nitroaromatic chemical is absorbed. In a study on the metabolism of 2,3,4,5- and 2,3,5,6-tetrachloronitrobenzene in rabbits (Bray *et al.*, 1953) it was noted that these compounds had very low water solubility and were only partially absorbed from the intestine. In this case, bacterial reduction prior to absorption may have accounted for most of the reduced metabolites identified. On the other hand, nitroaromatic compounds which are quickly absorbed would avoid extensive bacterial reduction. A study on the metabolism of the herbicide trifluralin in rats and dogs revealed that a large portion of the dose was excreted in the feces as reduced amino metabolites (Emmerson and Anderson, 1966). These results suggested that reduction had taken place in the intestine prior to absorption (Scheline, 1968).

Bacteria of the rumen are also known to reduce nitro groups. Golab and co-workers (1969) demonstrated that trifluralin labelled with ^{14}C when incubated with artificial rumen fluid, was rapidly transformed by reduction of both nitro groups (Figure 43). Subsequent investigations performed *in vivo* on a lactating cow confirmed the results of the *in vitro* assay.

An observation of the relative lack of toxicity of the pesticide parathion when given orally to cows was suggested to be due to nitro reduction by rumen bacteria to the corresponding amino compound (Cook, 1957).

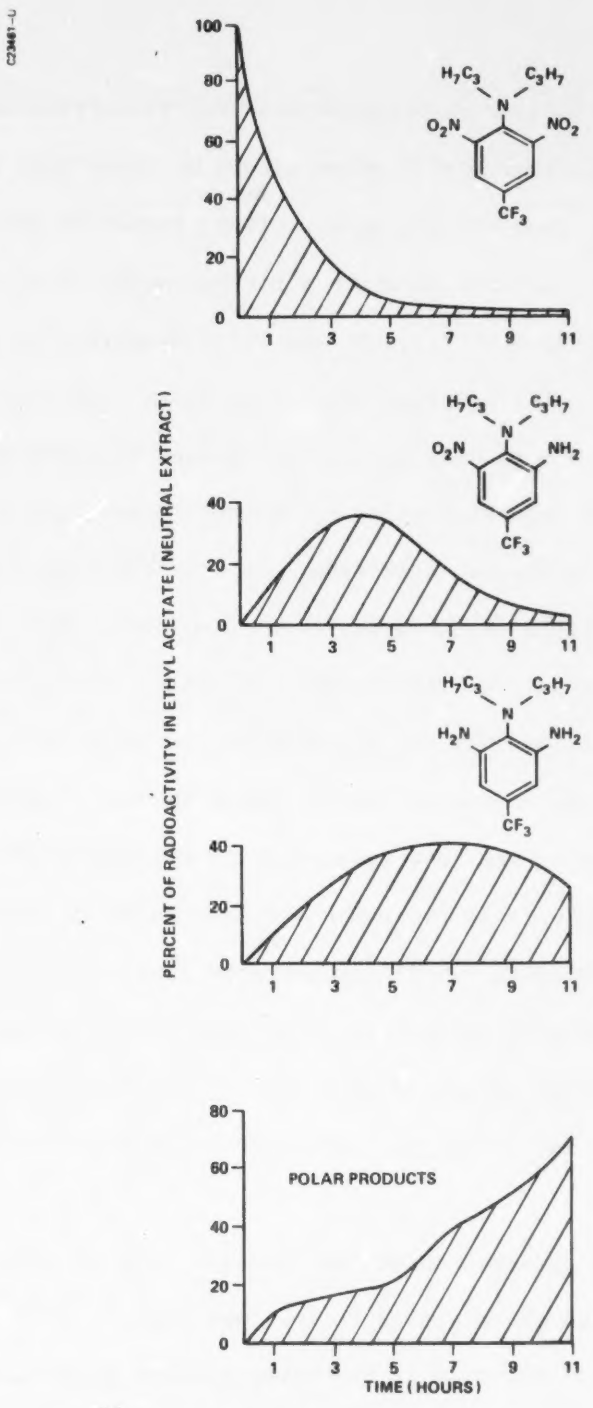


Figure 43. Rate of ^{14}C-Trifluralin Degradation and Formation of Degradation Products in Artificial Rumen Fluid (Golab et al., 1969)

Recent evidence has been presented which indicates that intestinal microflora may play a major role in the metabolic reduction of p-nitrobenzoic acid (PNBA) (Wheeler et al., 1975). The authors established that the anaerobic flora of the rat cecum was capable of reducing PNBA to p-aminobenzoic acid (PABA) by incubating rat cecal contents with PNBA under various conditions (Figure 44). Destruction of the microflora by autoclaving or the use of cecal contents from germfree rats resulted in the loss of PNBA reducing activity.

Noting that the majority of the flora of a rat is contained in the cecum, Wheeler and coworkers (1975) compared the reduction of PNBA *in vivo* using both conventional and cecectomized rats. They found that conventional rats were able to convert more than 20 percent of PNBA to PABA, whereas rats converted only 7 percent of the same dose when it was administered one week after cecectomy (Table 72). There was some evidence, however, of regaining the ability to reduce PNBA after cecectomy, as evidenced in rats fed PNBA 14 days after surgery.

These results cannot characterize the extent to which mammalian enzymes participate in the reduction of nitro groups, nor can they identify a possible joint participation of the liver and other organs with bacteria in metabolic nitro reduction. It is known, however, that induction of microsomal enzymes by DDT or phenobarbital increases PNBA reduction *in vitro* but not *in vivo* (Carlson and DuBois, 1970). Furthermore, the depression of xanthine oxidase function, an enzyme which reduces p-nitrobenzenesulfonamide in rat liver, causes a decreased rate of PNBA reduction *in vitro* but does not diminish the reduction of p-nitrobenzenesulfonamide *in vivo* (Westerfeld *et al.*, 1956). Wheeler and his associates (1975) found that the reduction of *p*-nitrobenzenesulfonamide when fed in the diet of rats occurred at the rate of 5 to 6% in germfree animals, compared to 46 to 47% in conventional rats. These results strongly suggest an important role of the microflora in the metabolic reduction of nitro compounds in general.

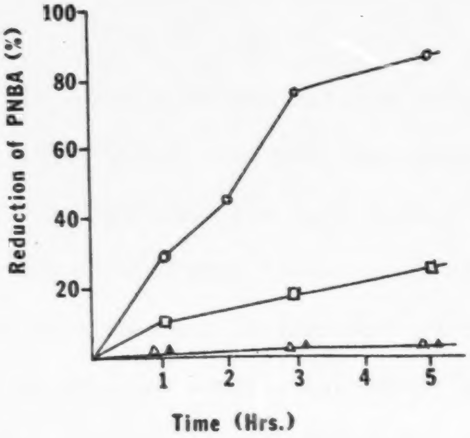


Figure 44. Typical Time Course of Reduction of PNBA by Rat Cecal Contents (from Wheeler *et al.*, 1975)

One milliliter of rat cecal contents (diluted 1:10 in sterile 0.1 M potassium phosphate buffer at pH 7.4) was added to 8.2 ml of this sterile buffer. The reaction mixture, containing 100 μg/ml of PNBA, was incubated at 37°C under sterile conditions. Aliquots were removed and assayed for PABA at the times shown and these data were plotted in terms of the conversion of PNBA to PABA. Cecal contents of conventional rats, incubated anaerobically (○) or in air (☐). Autoclaved cecal contents of conventional (or germfree) rats incubated anaerobically or in air (△). Cecal contents of germfree rats incubated anaerobically or in air (▲).

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Table 72. The Reduction of PNBA by Conventional and Cecectomized Rats[a] (Wheeler *et al.*, 1975)

Type of Rat	No. of Rats	Urinary Excretion Total PABA	 Recovery of PNBA and its metabolites[b]
		% of dose	
Conventional	5	20 (18-23)[c]	76 (72-85)
Conventional[d]	2	26, 25	
Cecectomized[e]	3	7.0 (6-8)	85 (40-110)
Cecectomized[f]	3	13 (9-19)	86 (70-97

a. PNBA (25 mg) was given orally unless otherwise noted. Urine was collected for 24 hours and analyzed.

b. Includes PABA and its conjugates (Total PABA).

c. Numbers are the average recovery of metabolites; the range is shown in parentheses.

d. PNBA (25 mg) given subcutaneously.

e. PNBA was fed seven days after the cecectomy operation.

f. Rats were refed PNBA 14 days after cecectomy.

4. Metabolic and Pharmacologic Effects

The nitroaromatic compounds as a group exert a varied and profound effect on metabolic and physiologic processes. Individual compounds in this group can cause severe hematologic changes including methemoglobinemia, sulfhemoglobinemia, Heinz body formation, and red cell destruction resulting in anemia. Certain nitroaromatics are unique in their ability to "uncouple" oxidative phosphorylation by suppressing the coupling of electron flow to synthesis of ATP. Several nitroaromatic chemicals cause allergic contact dermatitis, and one of these compounds, 2,4-dinitrochlorobenzene, is among the most potent primary skin sensitizers known. The major organs of foreign compound metabolism and detoxification, principally the liver and kidneys, are adversely affected by excessive exposure to any of the nitroaromatic compounds, and irreversible cellular damage may occur.

a. Hematologic Effects

Methemoglobinemia is probably the most notable manifestation at the biochemical level resulting from exposure to most of the nitrobenzene derivatives. The production of methemoglobin, a chemical analog of normal blood hemoglobin, results from an oxidation of the heme moiety of the molecule from its usual Fe++ (ferrous) state to an abnormal Fe+++ (ferric) state (Bodansky, 1951; Kiese, 1966; Nakajima and Kusumoto, 1963). In contrast to normal hemoglobin, which functions as an oxygen transporter to body tissues, methemoglobin binds oxygen so firmly that it cannot be released to the cells which require it.

Methemoglobin is normally present in the body at low concentrations and exists in equilibrium with normal hemoglobin. It is continually reduced by a methemoglobin reductase, diaphorase (Smith, 1969), and is thereby prevented from reaching excessive levels. Large amounts of methemoglobin produced in response to exposure to nitroaromatic chemicals will overload this reducing mechanism and diminish the oxygen-carrying capacity of the blood. This occurs not only by irreversible binding of oxygen to methemoglobin but also by interference with the release of oxygen from normal hemoglobin. Tissue hypoxia and cyanosis (bluish discoloration of the skin and mucous membranes) become evident when the oxygenated hemoglobin level falls below the critical cellular demand for oxygen.

The nitroaromatic compounds themselves are not generally regarded as direct methemoglobin producers, but rather their corresponding reduced metabolites are considered to be the active proximate agents (Linch, 1974). The hydroxylamine analogs of nitrobenzene, p-nitrotoluene, and p-nitroacetophenone were administered to mice and found to be active methemoglobin-inducing agents (Smith et al., 1967). These results, summarized in Table 73, demonstrated that p-hydroxylaminoacetophenone (p-HAAP), phenylhydroxylamine (PHA), and p-hydroxylaminotoluene (p-HAT) were all quite similar in their methemoglobinemia-inducing properties. The authors noted that

Table 73. Per Cent Circulating Methemoglobin at Various Times After the Injection of Aromatic Hydroxylamines in Female Mice* (From Smith et al., 1967)

Compound	10 min	20 min	40 min	60 min
p-HAAP	38.3 ± 5.8	32.8	6.2	0.7
PHA	42.1 ± 4.3	28.9	8.4	3.6
p-HAT	33.4 ± 5.7	18.5	5.1	2.3

* All chemicals given i.p. in propylene glycol, 0.1m-mole/kg. Values are either mean ± S.D. for six animals or the simple average for three. Ten-minute values for PHA and p-HAT are significantly different ($P<0.01$).

methemoglobinemia produced by PHA _in vivo_ was more short-lived than that produced by treatment of mouse red blood cells _in vitro_. This observation suggested a methemoglobin-reducing process which is external to the red cell and is functional only in the intact animal.

Metabolic reduction of the nitrophenols or aniline produces the corresponding aminophenol without necessarily forming a hydroxylamine intermediate. The ability of aminophenol to produce methemoglobin _in vivo_ was studied in female mice (Smith _et al_., 1967). Table 74 shows that, while _o_-aminophenol was considerably more active than _p_-aminophenol, it was nevertheless about tenfold less active than PHA. This observation supports the general assumption that the nitrophenolic compounds are not potent methemoglobin-inducers.

Table 74. Per Cent Circulating Methemoglobin at Various Times After the Injection of Aminophenols in Female Mice* (From Smith _et al_., 1967)

Isomer	10 min	20 min	40 min
_p_-Aminophenol	7.2	4.9	2.3
_o_-Aminophenol	32.6 ± 5.2	11.9	1.4

* Compounds given i.p. in aqueous solution, 1.0 m-mole/kg. Values shown are either mean ± S.D. for six animals or the simple average for three.

The supposition that nitroaromatic compounds must be reduced before they can produce methemoglobin has not been totally substantiated. One recent study (Kusumoto and Nakajima, 1970) indicated that methemoglobin could be formed _in vitro_ by incubation of nitrobenzene with human and rabbit hemoglobin. As shown in Figure 45, nitrobenzene converted a relatively small fraction (15%) of the hemoglobin to methemoglobin. This action was considerably less potent, however, than that produced by aminophenol incubated under similar conditions. Evidence was obtained in this study which demonstrated that nitrobenzene caused a structural change of hemoglobin protein which was similar in nature but less than that caused by aminophenol.

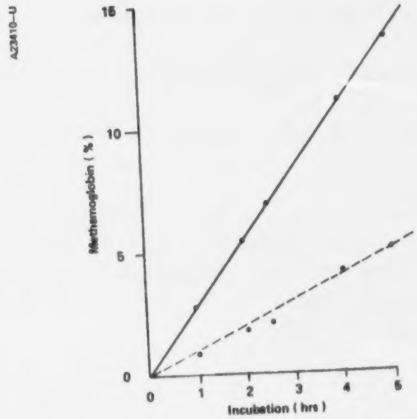


Figure 45. Methemoglobin Formation by Nitrobenzene In Vitro (Kusumoto and Nakajima, 1970)
(Final concentration, hemoglobin, 0.3 mM; nitrobenzene, 5 mM)
(●———●, with nitrobenzene; 0------0, without nitrobenzene)

Most recently, Brewer and Carr (1974) have also reported that incubation of human red blood cells with nitroaromatic compounds resulted in the direct production of methemoglobin. This activity was found to be related to the corresponding Hammett constant for the various nitrobenzene derivatives, with certain disubstituted compounds being even more potent.

Another hematologic indication of exposure to nitroaromatic compounds is the presence of Heinz bodies in the blood, usually accompanied by methemoglobinemia (Hanley and Mauer, 1961). These bodies are characterized as protein inclusion granules seen in the erythrocytes during poisoning by aromatic amines, nitro compounds, and nitrates and nitrate esters. The nature of this protein is not known, but it may be a denatured globulin. Heinz bodies are readily observed on staining with vital dyes and appear as dark particles in the bright yellowish-red erythrocytes. Their detection can serve as a valuable aid in the diagnosis and confirmation of hematologic poisoning. Table 75 illustrates the large number of Heinz bodies that can be formed within a very short time from administration of a nitroaromatic substance. Many investigators have found methemoglobinemia to be a variable

Table 75. The Occurrence of Heinz Bodies in the Peripheral Blood of Rabbits Following the Administration of a Single Oral Dose of β-Nitronaphthalene (BNN) (Dosage of BNN: 0.94 to 4.7 gm/kg) (From Treon and Cleveland, 1960)

Percentage of Erythrocytes Containing Heinz Bodies		Time After Administration of BNN (Days)	No. of Rabbits Examined
Average	Range		
0	0	0	7
0.75	0- 2	0.18	4
0.80	0- 4	0.25	5
8.5	7-10	0.32	2
33.0	32-34	1.0	2
87.6	63-98	1.25	7
90.6	75-98	2	7
88.4	85-95	3	7
81.5	75-89	4	6
7.7	6-13	8	3
7.0	-	9	1
7.0	-	11	1
8.0	-	15	1

response at best to nitroaromatic exposure in certain animals and man. The mouse is known to be particularly resistant to methemoglobin formation, presumably due to its very efficient methemoglobin-reducing ability (Smith *et al.*, 1967). The production of Heinz bodies, however, can be a much more sensitive indicator of intoxication and one which is usually observed in cases of nitroaromatic poisoning.

Hasegawa and Sato (1963) investigated the formation of methemoglobin and Heinz bodies in the blood of rabbits poisoned with *p*-chloronitrobenzene. Twenty-four hours after subcutaneous administration of 500 mg/kg body weight, each erythrocyte of the rabbit contained one Heinz body. After 48 hours, multiple Heinz bodies were observed both inside and outside the red blood cells. The ratio of methemoglobin to total hemoglobin rapidly increased upon injection of the chemical, then leveled somewhat after seven hours, thereby indicating a biphasic formation process (Figure 46).

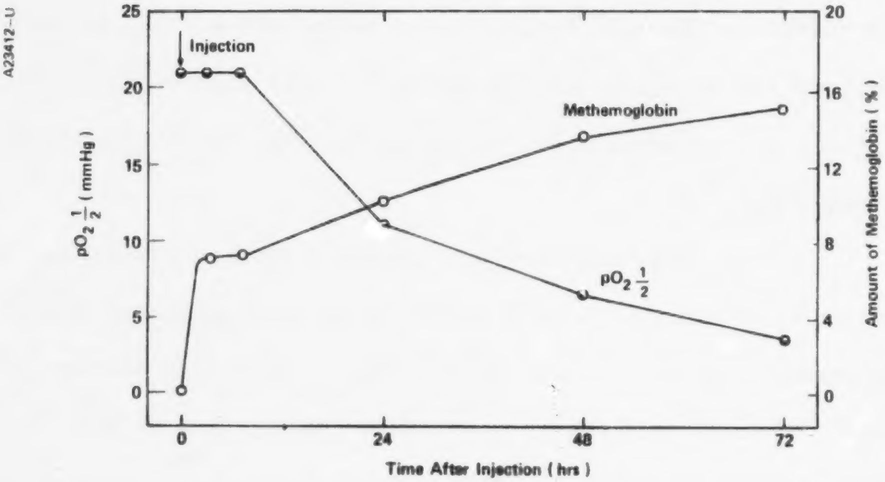


Figure 46. Plots of the Amount of Methemoglobin and Oxygen Affinity of Hemoglobin ($pO_{2\frac{1}{2}}$) versus Time After Injection (Hasegawa and Sato, 1963)

The authors cited previous studies that confirmed this process and noted that methemoglobin produced in the initial stage was more easily reduced to normal hemoglobin than that which was formed later. An analysis of hemoglobin isolated from poisoned rabbits indicated that a functional change had taken place which greatly increased its oxygen affinity with increasing time after injection of p-chloronitrobenzene. This change, of course, has serious physiologic consequences. They found that, 72 hours after treatment, the hemoglobin of poisoned rabbits was 95 percent oxygenated, where normally one third of the oxygen from oxyhemoglobin should have been removed. This would cause a severe deprivation of oxygen to body tissues due to the increased strength of its bond with circulating hemoglobin.

The appearance of sulfhemoglobin in the blood following exposure to nitroaromatics usually parallels the production of methemoglobin, but sulfhemoglobin is much more persistent. It is probably produced as an intermediate of bile pigment formation from hemoglobin. Sulfhemoglobin was found to remain in the body for more than three months after formation, and the length of its retention is related to the life-span of the red blood cell (116 days). Methemoglobin, on the other hand, is usually eliminated in two to five days.

The pharmacologic effect of several nitroaromatic derivatives on blood platelet levels was found to be significant only in the case of o-nitrophenol (Gabor et al., 1962). When 31 rats were administered o-nitrophenol by intraperitoneal injection at 1 mg/kg body weight, the platelet count was observed to increase significantly (Figure 47). Even at doses of 0.1 mg/kg body weight, a similar effect was produced. The administration,

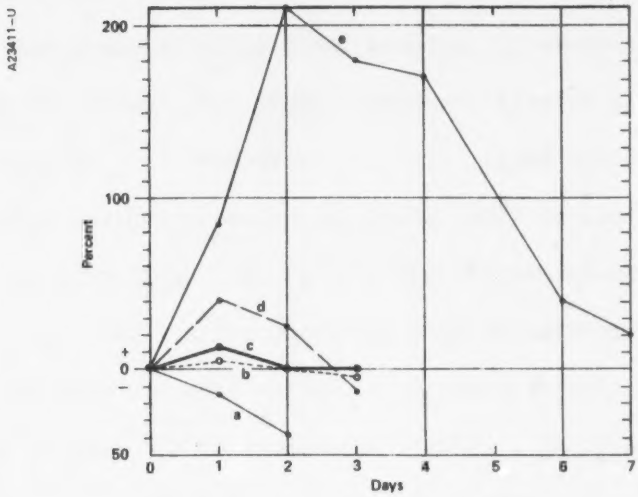


Figure 47. The Platelet-Count of Rats After the Administration of Phenol Derivatives. a 2,4-dinitrophenol; b m-aminophenol; c p-aminophenol; d m-nitrophenol; e o-nitrophenol. Dose: 10 mg/100 g i.p. (Gabor et al., 1962)

however, of 2,4-dinitrotoluene, nitrotoluenes, 2,4-dinitrophenol, or p- and m-nitrophenol did not produce a rise in platelet levels. Additional data are not available to explain this unique phenomenon.

b. Skin Sensitization

Several halogen-substituted nitrobenzene derivatives have been demonstrated to produce allergic contact dermatitis. Most notably, 2,4-dinitrochlorobenzene (DNCB) is an extremely potent skin sensitizer in both animals (Polak and Frey, 1974) and man (Catalona et al., 1972a, 1972b; Malaviya et al., 1973; Lowney, 1971).

Contact sensitization to DNCB results from its ability to act as a hapten when topically applied and to form covalent bonds with lysine groups of epidermal proteins (Eisen et al., 1952; Eisen and Tabachnick, 1958;

Nakagawa et al., 1971). Sensitization occurs in greater than 95 percent of those exposed, taking place in regional lymph nodes (Kligman and Epstein, 1959).

An allergic response occurs when sensitized lymphocytes contact the protein-bound DNCB. Circulating (humoral) antibodies do not participate in reactions to DNCB, since the process involves only cell-mediated immunity. Sensitization may take place 7 to 21 days after DNCB exposure and will be manifested by a spontaneous flare reaction at the site of application, resulting from minute concentrations of bound DNCB remaining in the skin (Catalona et al., 1972a). Those who do not respond spontaneously to DNCB application can be made to exhibit the allergic response by reapplication of a weak solution of DNCB.

In addition to being a potent skin sensitizer, DNCB is also an extremely strong primary skin irritant. Catalona et al. (1972a) reported that doses normally used for sensitization, ranging from 1000 to 2000 μg, would cause erythema and edema within 12 hours, followed by desquamation and pigmentation after several days.

Allergic sensitization of the skin can also be achieved by contact with 2,4-dinitrofluorobenzene (DNFB). DNFB acts in a similar fashion to DNCB, combining with epidermal and dermal proteins to form an antigenic hapten-carrier complex. Schneider (1974) described the so-called "blastogenic response" to the application of DNFB on mice whereby lymphocytes migrate to the regional lymph node draining the site of sensitization and become blast cells. These blast cells rapidly proliferate for several days and then disappear from the lymph node after four or five days. These cells apparently enter the circulation and bone marrow to function as memory cells to maintain the state of contact sensitivity to future applications of DNFB.

The monochloronitrobenzenes, specifically the *ortho*- and *para*-isomers, are allergic skin sensitizers in rats and guinea pigs (Rusakov *et al.*, 1973). Neither compound, however, is as strong an allergen as DNCB.

c. Uncoupling of Oxidative Phosphorylation

One of the most outstanding features of exposures to nitroaromatic compounds is the relationship of the dinitrophenols to bioenergetics. The oxidation of foodstuffs to produce energy and numerous biosynthetic oxidation-reduction and oxidative phosphorylation reactions are essential to the maintenance of life. The presence of dinitrophenol compounds in biological systems causes severe physiologic disturbance by the uncoupling of oxidative phosphorylation.

Oxidative phosphorylation and the electron transport sequence in aerobic organisms account for the reoxidation of reduced nicotinamide or flavin nucleotides and subsequently the generation of adenosine triphosphate (ATP) by phosphorylation of adenosine diphosphate. The amount of ATP available to the cell is critical in providing adequate energy sources for metabolic and biosynthetic reactions. The oxidation of the nicotinamide and flavin coenzymes is necessarily coupled to phosphorylation. Figure 48 portrays the scheme for reoxidation of the nicotinamide coenzymes. The flavin coenzymes may also be reoxidized by the electron-transport sequence by entering the reaction at the point of CoQ.

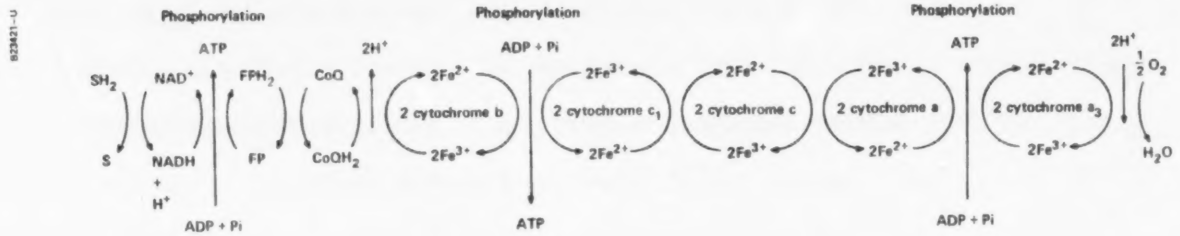


Figure 48. The Electron Transport Sequence and Probable Sites of Coupled Oxidative Phosphorylation (This diagram shows for example that 2 molecules of reduced cytochrome b react with 2 molecules of oxidized cytochrome c_1, and this oxidation-reduction process is coupled with the transformation of ADP to ATP.) (From Feuer, 1970)

The uncoupling action of 2,4-dinitrophenol results in the reoxidation of the reduced coenzymes without the concomitant production of ATP (Feuer, 1970). This uncoupling effect will prevent the utilization of foodstuffs and deprive tissues of the energy needed for muscular work and biosynthetic reactions. Because the coenzymes are reoxidized and used to further metabolize foodstuffs, but no ATP is generated, the organism can take in large quantities of nutrients and yet starve, due to a lack of biochemically useful energy.

Several theories have been advanced concerning the underlying mechanism of this uncoupling effect by dinitrophenol. Weinbach and Garbus (1969) believed that the ability of uncoupling agents to combine with proteins is a key to their action. Based upon their investigations, the authors concluded that uncoupling reagents can bind to mitochondrial proteins, as well as to soluble cellular and serum proteins. In their laboratory, it was demonstrated that the halo- and nitrophenols interact with mitochondrial proteins to produce conformational changes which were thought to result in profound changes in their biological activity, and hence to form the basis for the uncoupling phenomenon.

A proposed sequence of events in the uncoupling of oxidative phosphorylation would be: 1) the entrance of the undissociated reagent into the mitochondria followed by ionization of the compound and interaction with charged ε-amino groups of the protein, 2) reorganization and conformational transition of protein structure leading to, 3) alteration in the activity of enzymes catalyzing the coupling of phosphorylation to electron transport.

In the past, many investigators have attributed the uncoupling action of dinitrophenol to an acceleration of the hydrolysis of oxidative phosphorylation intermediates (Ernster and Lee, 1964). Studies by Pinchot (1967), however, disagree with this theory. He suggested that phosphorylating particles containing a coupling enzyme formed a high energy intermediate of oxidative phosphorylation. Dinitrophenol interfered with the ability of the enzyme to bind with the electron transport particle and thereby inhibited the oxidative phosphorylation process.

Taken together, the above studies, while not providing a single definitive explanation for the mechanism of action of dinitrophenol, provide strong evidence for disruption of a catalytic enzyme or enzyme complex essential to the oxidative phosphorylation process.

In addition to the dinitrophenols and dinitrocresols, several of the nitrosalicylanilides are known to be among the most effective uncouplers of oxidative phosphorylation ever tested (Williamson and Metcalf, 1967). An examination of the structural similarities of three potent aromatic uncouplers, including a nitrosalicylanilide (Figure 49), reveals that strong electron-withdrawing groups are positioned at a fixed distance from the halogenated aryl ring. The mode of action for these compounds in uncoupling oxidative phosphorylation, as suggested by their common structural features, may be a preferential adsorption to an active enzyme site.

Bachmann *et al*. (1971) have reported that 2,6-dichloro-4-nitroaniline (DCNA) is also an effective uncoupler of oxidative phosphorylation. At a concentration of 5×10^{-5}M with rat liver mitochondria *in vitro*, DCNA uncoupled oxidative phosphorylation and inhibited mitochondrial electron transport. Dinitrophenol was similarly effective at a concentration of 1×10^{-5}M. When administered orally to rats for four days at 1000 mg per kg body weight per day, DCNA caused an inhibition of oxidative phosphorylation in isolated mitochondria.

These authors have related the uncoupling potency of DCNA, and phenolic compounds in general, to their degree of dissociation and lipid solubility. Critical factors appear to be the ease with which a compound can enter the mitochondria and its capacity to form a phenolic anion which can interact with an electron-deficient enzyme of the phosphorylation reaction chain. Alternatively, evidence has been presented (McLaughlin, 1972) to show that the anion of dinitrophenol can bind to mitochondrial membranes and produce a substantial negative surface potential. The significance of this effect to the uncoupling phenomenon is not entirely clear. Additional data have been provided (Verma *et al*., 1973) which indicate that uncouplers can change the organization of phospholipid multibilayers. It was suggested that these changes

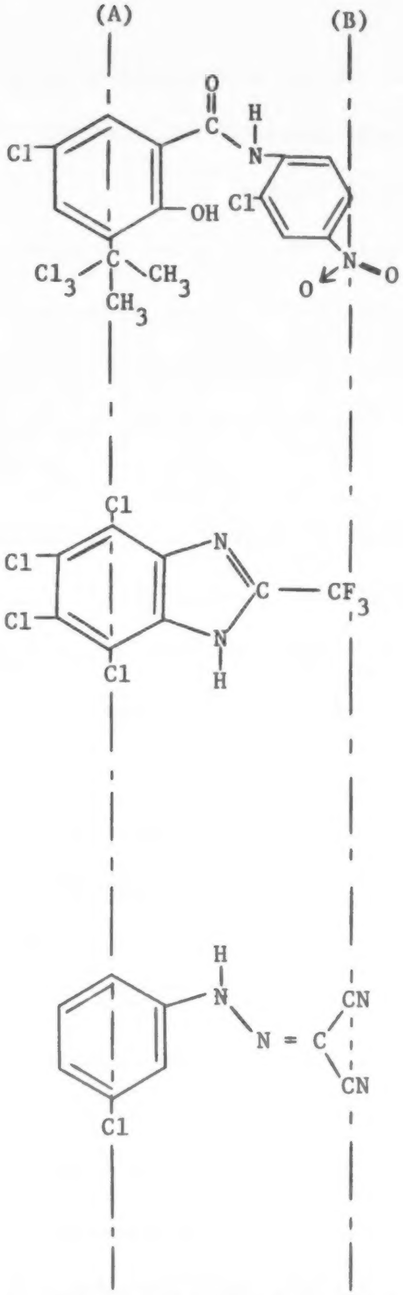


Figure 49. Structural Similarities Among Various Uncouplers of Oxidative Phosphorylation. (A) Represents the plane of symmetry through the halogenated aromatic rings and (B) is the corresponding plane of symmetry through the electron withdrawing groups. (Williamson and Metcalf, 1967)

could alter the properties of lipid-bound membrane enzymes involved in oxidative phosphorylation and thereby uncouple the system.

(i) Metabolic Disruption

Metabolic effects which accompany the uncoupling of oxidative phosphorylation are increases in body temperature, respiration rate, oxygen consumption, glycogenolysis, and thyroxin secretion, as well as the production of hypertension and effects on neuromuscular transmission and bile secretion.

Hyperthermia (hyperpyrexia, elevated body temperature) by the action of uncoupling chemicals results from increased heat production rather than decreased heat loss. Gatz and Jones (1970) explained that high phosphate bond energy normally captured in the production of ATP is lost as heat when oxidation is uncoupled from phosphorylation. They found that the time of onset and degree of dinitrophenol-induced hyperthermia were exponentially dose-related when the compound was administered intraperitoneally to rats (Figure 50). The authors hypothesized that a 10 percent uncoupling of oxidative phosphorylation would increase heat production by 65 to 100 percent, and a 20 percent uncoupling would raise heat production by 275 to 425 percent.

In studies by Hull _et al_. (1971), 5 mg per kg body weight of dinitrophenol injected intravenously in dogs produced little temperature change. The same dose, however, administered 20 minutes after the induction of general anesthesia by halothane produced a dramatic and lethal rise in temperature.

Similarly, Hoch and Hogan (1973) measured the metabolic rate in rats treated with halothane and dinitrophenol, both alone and

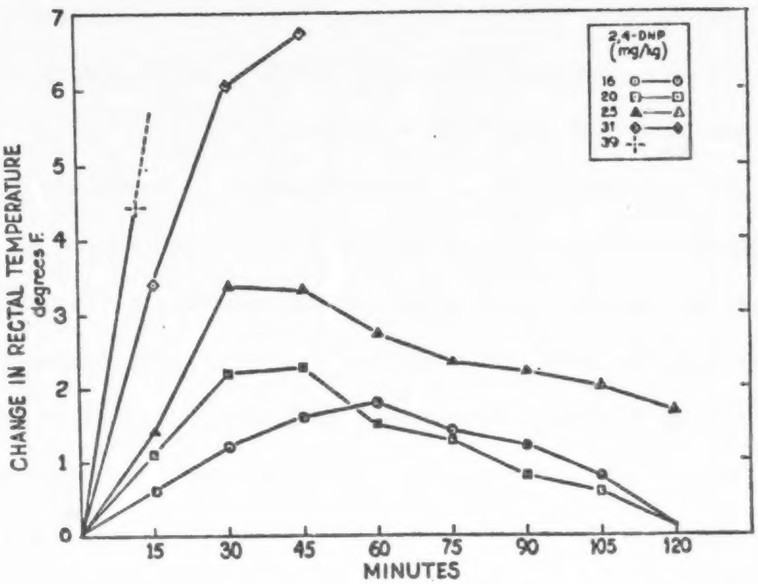


Figure 50. Effect of Logarithmically Increasing Intraperitoneal Doses of 2,4-DNP on Rectal Temperature in Rats. Each point on a curve represents the mean of between 6 to 8 determinations of temperature at the specified times. (Gatz and Jones, 1970)
(Reprinted with permission from the International Anesthesia Research Society.)

in combination. The intraperitoneal injection of 10 mg per kg body weight of dinitrophenol alone promptly doubled the metabolic rate and increased body temperature by 10 percent. Gradual recovery and no mortality resulted following this treatment. In combination with halothane, dinitrophenol evoked an additional sharp rise in temperature of about four degrees C. and a tripled metabolic rate. Death followed within two to seven minutes of the sharp temperature rise.

The above cases of hyperthermia typify the hazards of synergistic reactions posed by substances which probably act in a similar fashion on the body. Since dinitrophenol, and presumably halothane, can both act on the cellular mitochondria to disrupt oxidative phosphorylation, subthreshold doses of each compound when combined may produce severe physiological disturbance through additive action at a common site.

The effect of dinitrophenol in stimulating the conversion of glycogen to glucose is due, in part, to its action on the phosphorylase enzyme system. Studies by Vercesi and Focesi (1973) and Focesi _et al_. (1969) established that dinitrophenol could markedly increase the level of phosphorylase b kinase in skeletal and cardiac muscle of the rat. Phosphorylase b kinase is an enzyme which catalyzes the conversion of inactive phosphorylase b to the activated enzyme form phosphorylase a. The relationship of the phosphorylases system to glycogenolysis is explained by Segal (1973) and illustrated in Figure 51. Glycogenolysis is normally stimulated by

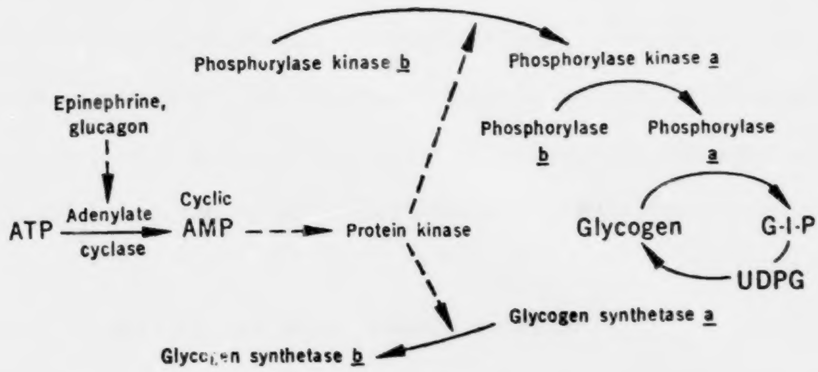


Figure 51. Epinephrine and Glucagon Control of Glycogen Metabolism (Segal, 1973)
(Reprinted from _Science_ (1973), H.L. Segal, _180_, 25-32. Copyright 1973 by the American Association for the Advancement of Science.)

the hormones glucagon and epinephrine via the conversion of phosphorylase b to a through the mediation of cyclic AMP. In the case of dinitrophenol poisoning, however, it may be possible to elevate cyclic AMP levels without hormonal stimulation.

In skeletal muscle, dinitrophenol administered at 25 mg per kg body weight in rats produced a threefold increase in phosphorylase a levels. Similarly, in cardiac muscle of the rat the same dinitrophenol treatment tripled phosphorylase a content and more than doubled the level of phosphorylase b kinase (Table 76).

Table 76. Content of Phosphorylase a and Total and of Phosphorylase b Kinase in Hearts of Rats Poisoned With DNP 2.5 mg/100 g

(From Vercesi and Focesi, 1973)

Determination	Normal Rats	DNP poisoned rats
Phosphorylase a (10 rats)	7.0 ± 1.7[a]	25.2 ± 3.1
Total phosphorylase (+AMP) (10 rats)	67.2 ± 7.1	85.3 ± 13.0
Ratio Phosphorylase a/Total Phosphorylase X 100	10.4 ± 1.1	30.7 ± 6.5
Phosphorylase b kinase (10 rats)	1346 ± 480	3384 ± 334

[a] S.E.M. The activity of phosphorylase a and total are expressed in units according to Cori et al. (1955) per gram of heart. The activity of phosphorylase b kinase is expressed in units of phosphorylase a formed from phosphorylase b in 15 min according to Fischer and Krebs method (1962).

In studying the effects of dinitrophenol on pulmonary function in dogs, Cardus and Hoff (1963) measured oxygen consumption, ventilation, and respiratory frequency. They found that intravenous injection of dinitrophenol at doses of 3, 6, or 9 mg per kg body weight invariably produced elevations in rectal temperature within five to ten minutes after injection, and a rapid increase in oxygen consumption (Figure 52). Increases in the frequency and amplitude of respiratory movements were seen at the two higher doses. In all dogs, there was an effect on the ventilation within 20 seconds after injection of the drug. Sudden increases in respiratory frequency and amplitude which lasted for about 10 seconds were observed, followed by reestablishment of the control pattern. A more gradual pattern of rise in frequency and amplitude was seen thereafter in the 6 and 9 mg per kg groups.

The authors noted that the correlation between ventilation and rectal temperature was considerably greater than the correlation between ventilation and oxygen consumption. The relation between dinitrophenol and increased ventilation could only be explained by an indirect action on ventilation by a primary change in temperature or by a direct action on some unknown receptor which elicits an independent respiratory response.

An earlier study by Harvey (1959) measured the effect of 4,6-dinitro-o-cresol on oxygen consumption in guinea pigs. Similar to the case of dinitrophenol in dogs, dinitro-o-cresol produced a marked rise in oxygen consumption from 6 to nearly 100 percent when doses of 5 to 20 mg per kg were administered (Figure 53).

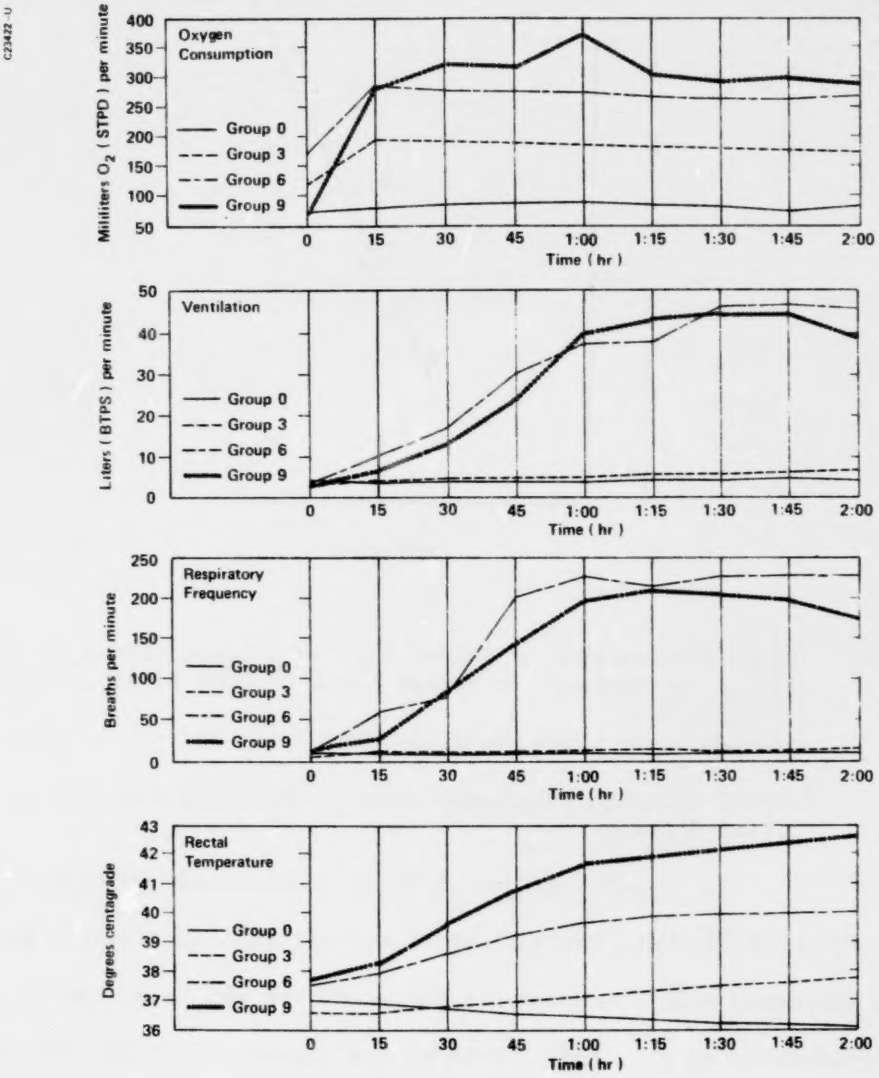


Figure 52. Response to Intravenous Dinitrophenol Injection in Dogs* (Cardus and Hoff, 1963)

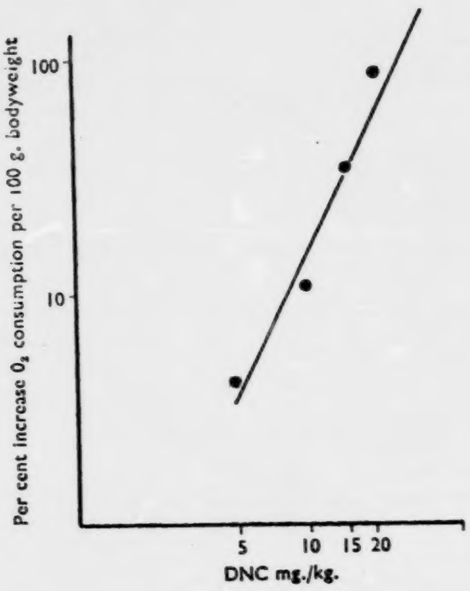


Figure 53. Oxygen Consumption of Guinea Pigs -- Average Weight of Group 1500 g, in Response to Varied Doses of DNOC Given Intraperitoneally (Harvey, 1959)
(Measurements taken 1.0-1.25 hours after injection. Line by observation.)
(Reprinted with permission from the Pharmaceutical Society of Great Britain.)

The nature of dinitrophenol-induced changes in thyroid function has been the subject of several investigations. Early studies indicated that dinitrophenol depressed the uptake of iodine into the thyroid (Goldberg et al., 1955; Freinkel and Ingbar, 1955) and lowered the plasma protein-bound iodine (Wolff et al., 1950). Dinitrophenol-induced changes in pituitary histology have also been reported (Goldberg et al., 1957).

In a study where human volunteers were each fed 225 mg of dinitrophenol per day for two consecutive days, a significant decrease in protein-bound iodine and a rise in the metabolic rate were observed (Castor and Beierwaltes, 1956). Changes were not seen either on thyroidal I^{131} uptake and release, or on the excretion of I^{131} in urine and feces. The authors postulated that the mechanism of dinitrophenol action in lowering protein-bound iodine was due to an increased utilization of the thyroid hormone.

Later studies (DeFelice and Rupp, 1963; Reichlin, 1960) indicated that altered thyroid function by dinitrophenol may be due to an inhibition of the production or release of thyroid-stimulating hormone in the pituitary. Maayan (1968) demonstrated that the thyroidal growth response to thyroid-stimulating hormone was blocked by dinitrophenol and accompanied by a decreased uptake of I^{131} into the thyroid.

Most recently, England _et_ _al_. (1973) have established that dinitrophenol in fact increases thyroid output and thyroid hormone excretion in the bile, while at the same time lowering the plasma protein-bound iodine. They suggested that the mechanism of action for dinitrophenol on thyroid hormone metabolism may be a competition for thyroid hormone binding sites on plasma proteins leading to a fall in protein-bound iodine concentration. Consequently, newly formed thyroid hormone would be displaced from the plasma and wasted by excretion in the bile. This wastage would serve to increase demand via the pituitary feedback system for thyroid hormone. As a result, thyroid-stimulating hormone secretion would rise, and subsequently thyroid hormone output would be increased.

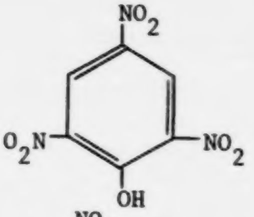
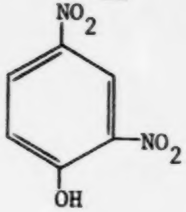
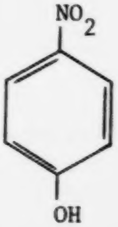
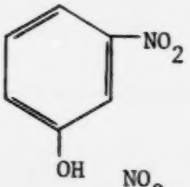
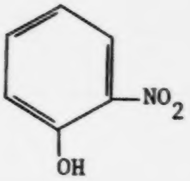
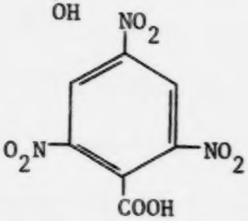
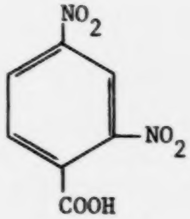
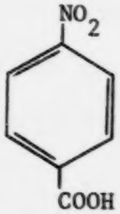
(ii) Neurologic Effects

The mode of action of nitroaromatic compounds on nervous transmission is not entirely clear at this point. It has been reported (Takagi and Takayanagi, 1965) that picric acid, 2,4-dinitrophenol, and all nitro-derivatives of benzoic acid induce the contraction of guinea pig small intestine by increasing the liberation of acetylcholine from cholinergic nerve endings. The mononitrophenol derivatives, on the other hand, had very low activity, and, in fact, inhibited the acetylcholine liberation induced by the active nitroaromatics. The concentrations of the compounds tested that produce contractions of smooth muscle of guinea pig small intestine are shown in Table 77. When applied to the rectus abdominus muscle of the frog, *p*-nitrophenol induced contraction by the liberation of acetylcholine. This finding suggested that the nerve ending of guinea pig small intestine behaved differently than that of skeletal muscle in the frog.

Studies were designed by Beani *et al*. (1966) to determine the effect of dinitrophenol *in vitro* on the neuromuscular junction of rat and guinea pig phrenic nerve-diaphragms. Their results indicated that dinitrophenol reduced acetylcholine release from electrically-stimulated diaphragms. Reduction of acetylcholine output corresponded with the depletion of tissue stores, thereby indicating a possible exhaustion of acetylcholine available for release (Table 78).

In studying the actions of dinitrophenol on neurons of the cerebral cortex, Godfraind *et al*. (1970) discounted the hypothesis of a primary metabolic action of acetylcholine. They found instead that dinitrophenol causes a profound decrease in electrical excitability associated with a

Table 77. Effective Concentration of Nitrocompounds Tested on the Small Intestine of Guinea Pig (Takagi and Takayanagi, 1965)

Compound	Effective concentration (g/ml)
	5 X 10^{-6} — 5 X 10^{-5}
	3 X 10^{-5} — 3 X 10^{-4}
  	(1)
	5 X 10^{-6} — 5 X 10^{-5}
	10^{-5} — 10^{-4}
	10^{-4} — 10^{-3}

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(1) Their intrinsic activity is low or zero, so they acted as an antagonist of the agonists listed in this table.

Table 78. Effect of 2,4-Dinitrophenol Upon Total Acetylcholine Content of Guinea Pig Hemi-Diaphragms (From Beani *et al.*, 1966)

Measurements were made at the end of the fourth period of stimulation at 6/sec, lasting 10 min; temperature 33°C, dyflos 500 μg/ml pretreatment. The % reduction of acetylcholine release during stimulation at 6/sec is given for comparison.
*; significantly different ($0.05 > P > 0.02$) from the control group.
**; significantly different ($P < 0.001$) from the control group.

DNP Concentration (M)	No. Expts.	Hemi-diaphragm weight ± S.D.	Acetylcholine ng/hemi-diaphragms ± S.D.	Acetylcholine stores %	Acetylcholine release %
-	16	274.0 ± 29.0	122.3 ± 28.0	100	100
3×10^{-6}	10	229.0 ± 38.0	103.1 ± 15.6	91.8	92.7
3×10^{-5}	10	255.0 ± 27.0	87.3 ± 24.0*	77.7	64.4
3×10^{-5}	12	276.0 ± 38.0	71.4 ± 12.8**	63.5	41.2

tendency to hyperpolarization and an increased permeability of the membrane to K^+. Similarly, Dimov *et al.* (1972) related the suppressive effect of dinitrophenol on cortical bioelectric activity to its penetration into the nervous cells. They found that dinitrophenol disrupted the development of an epileptogenic focus in the brain of cats and suggested that a certain "critical" level of ATP must be available for the induction of paroxysmal activity.

d. Organoleptic Properties

Studies from the Russian literature have been encountered which reported a threshold concentration for odor, taste, and color of several nitroaromatic compounds in reservoir waters. Makhinya (1964) reported that the threshold concentration of *o*-nitrophenol was 3.83 mg/ℓ for odor, 8.6 mg/ℓ for taste, and 0.6 mg/ℓ for color. Concentrations of p-nitrophenol were 58.3, 43.4, and 0.24 mg/ℓ for odor, taste, and color, respectively. The values for m-nitrophenol were given as 389, 164.5, and 26.3 mg/ℓ, respectively. In a later report (Makhinya, 1967) threshold limit concentrations for m-nitrophenol were given as 350.3 mg/ℓ for odor, 144.8 mg/ℓ for taste, and 26.3 mg/ℓ for discoloration of aqueous solutions.

In a similar study by Kosachevskaya (1967), the ortho-, meta-, and para-nitrotoluene isomers imparted a bitter taste and an odor of bitter almonds to water at concentrations of 0.01 to 0.2 mg/ℓ. The meta- and para-nitrotoluene isomers had the greatest effect upon taste (threshold value = 0.01 mg/ℓ); the limit value for ortho-nitrotoluene was 0.05 mg/ℓ.

C. Toxicity - Humans

There is little doubt concerning the potential for adverse human reaction when dealing with the nitroaromatic compounds. The danger from human exposure to these substances, even in small amounts, can range from the production of a mild and transient episode of cyanosis to a dramatic and sudden collapse culminating shortly in death. While it appears that a threshold limit of exposure exists, below which no toxic effects are manifested, chronic exposure to subacute doses can ultimately produce severe and irreversible physiologic damage. The greatest danger of poisoning generally exists among those persons who are involved in manufacture or who directly handle and use these compounds on a large scale.

Frequently, careless work habits or insufficient protection from chemical dusts and fumes have led to incidents of serious intoxication. A major factor in determining the severity and permanent damage resulting from nitroaromatic exposure has been the failure of the person exposed to recognize the symptoms of a toxic reaction and to act immediately in seeking medical care. Although the signs of poisoning can be quite varied for the different nitroaromatic compounds, the potential for serious illness is sufficient to warrant the recommendation of special monitoring measures in several industries. It is of primary importance in the treatment of occupational poisoning that the patient be removed from the workplace and that all contaminated clothing, including shoes and underwear, be removed immediately and the patient bathed. Absorption via the skin from contaminated clothing has often been found to result in chronic intoxication and relapses into acute poisoning.

The dangers of the nitroaromatic compounds have been commonly referred to since about the beginning of this century, and many texts dealing with toxic materials have devoted considerable discussion to their effect upon humans (Hamilton and Hardy, 1974; Moeschlin, 1965; Hunter, 1969; Arena, 1974). However, despite the fact that several hundred nitroaromatic compounds are produced commercially, documented reports of poisoning and assessment of health hazard potential have been limited to a relatively small number of these chemicals. Substances which have received the greatest amount of attention in the literature, due to their toxic nature and long history of varied use, include nitrobenzene, dinitrobenzenes, chloronitrobenzenes, nitrotoluenes, dinitrotoluenes, trinitrotoluenes, nitroanilines, nitrophenols, dinitrophenols, dinitrocresols, and tetryl.

The major toxic symptom from exposure to the above compounds, excepting the nitrophenol derivatives, is undoubtedly cyanosis and the production of methemoglobin. This response is often the only manifestation of exposure, and in most cases is rapidly reversible without specific treatment other than removal from exposure. Fatalities rarely occur by hematologic disturbance. Less common but more severe reactions may include liver damage, severe anemia, bone marrow changes, renal failure, and acute dermatitis. Dinitrophenol and dinitrocresol, on the other hand, can produce severe metabolic disturbances and death by respiratory paralysis.

A characteristic observation among cases of occupational poisoning is that the onset of acute symptoms often occurs several weeks or months after the last exposure to the substance, or after many months of continuous exposure on the job. The appearance of a toxic episode, however, can be sudden, extremely severe, and many times can end shortly in death. The ingestion of alcoholic beverages has been related to the precipitation of many cases of acute poisoning in persons who have handled nitrobenzene derivatives. This phenomenon is presumably due to a sudden "washing-out" effect of ethanol on these substances from fat deposits in the body where they are selectively accumulated.

1. Occupational Studies

Occupational exposure to the nitroaromatic compounds has provided numerous cases of poisoning and death. Prior to the institution of rigid industrial hygiene standards, fatalities due to nitroaromatic exposure were common, as indicated in a summary of the early literature by Von Oettingen (1941).

The outstanding manifestations of toxic exposure to most nitroaromatics are cyanosis, anemia, and the production of methemoglobin. The biochemical basis and mechanisms of methemoglobin formation have been discussed in Section III-B-4. A comprehensive medical surveillance program by Linch (1972, 1974) resulted in the evaluation of 187 cyanosis cases, diagnosed on the basis of laboratory findings, during the ten years from 1956 to 1966. He found that acute exposure produces cyanosis and possible loss of hemoglobin, while chronic subacute absorption may lead to reversible anemia. The relative cyanogenic and anemiagenic potentials of many of the nitroaromatics are presented in Table 79. Increased environmental temperature, in addition to chemical structure, was also shown to be a factor in cyanosis production (Figure 54).

Table 79. The Chemical Cyanosis Anemia Syndrome-Hazards of the Nitroaromatic Compounds (Linch, 1974)

Rank	Cyanogenic Potential	Anemiagenic Potential	Over-all Potential
1	dinitrobenzene	nitrobenzene	dinitrobenzene
2	m-nitroaniline	mixed-, p-nitrochlorobenzene	nitrobenzene
3	p-nitroaniline	mixed-nitrotoluene	mixed-, p-nitrochlorobenzene
4	nitroanilines	dinitrobenzene	nitroanilines
5	nitrobenzene	nitroanilines	mixed-nitrotoluene
6	o-nitrochlorobenzene	p-dinitrosobenzene	p-dinitrosobenzene
7	p-nitrochlorobenzene	nitronaphthalene	nitronaphthalene
8	mixed-nitrochlorobenzene		
9	p-dinitrosobenzene		
10	o-nitrotoluene		
11	p-nitrotoluene		
12	mixed-nitrotoluene		
13	dinitrotoluenes		
14	nitronaphthalene		

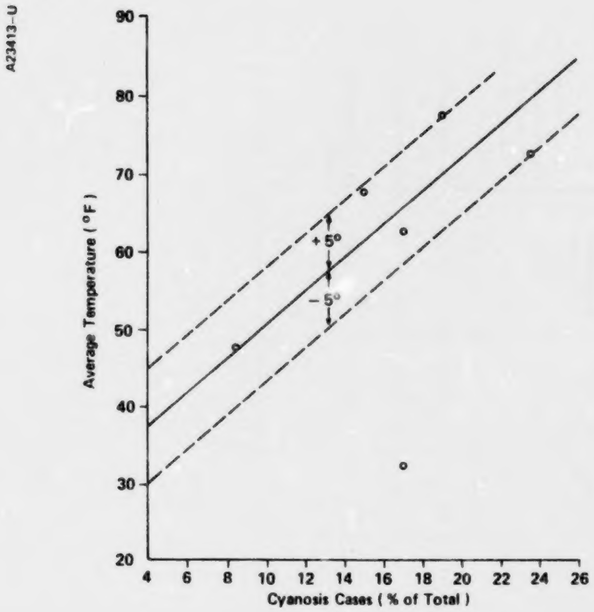

Figure 54. Effect of Temperature Upon Cyanosis Occurrence (Linch, 1972)

An early attempt was made by Pasceri and Magos (1958) to attach a diagnostic significance to quantitative differences in the levels of methemoglobin, sulfhemoglobin, and Heinz bodies in the blood of chemical production workers. Their investigation at eight industrial plants included air contamination studies, medical examination of workers, and urine testing for the presence of toxic chemicals and metabolites. The most common routes of exposure to nitroaromatic chemicals were found to be (1) inhalation and (2) skin contact resulting from hand-feeding and discharge of chemicals into processing vessels and cleansing and repair of containers. The results of biochemical determinations made on the blood of exposed workmen are presented in Table 80.

Table 80. Blood Examinations of Workmen in Aromatic Chemical Production Plants
(From Pasceri and Magos, 1958)

Plant	Compound	Test period	Number of Subjects	Anemization	Methemoglobin		Sulphhaemoglobin		Heinz body formation (above 1%) in percentage of tests	Notes
					average g. %	stand. dev.	average g. %	stand. dev.		
Anisidine production	nitroanisole anisidine	VI.-XII. 1954	23	some cases transitory	0.67	(±0.39)	0.32	(±0.18)	43.0*	*Individually 1-3%, except 2 cases, in which 5.7%
Phenacetine production	p-nitrochlorobenzene nitrophenetol phenetidine	IX.-XII. 1954	30	some cases transitory	0.78	(±0.37)	0.20	(±0.14)	10.0*	*Individually 1-3%
Production of nitroaromatic compounds	nitrobenzene p-nitrochlorobenzene dinitrochlorobenzene nitroethylbenzene	VIII.-XII. 1954	39	some cases transitory	0.61	(±0.38)	0.27	(±0.14)	2.8*	*Individually 1-3%

The authors established an average normal value for methemoglobin at 0.22 ± 0.14 g% (upper limit: 0.50 g%) and for sulfhemoglobin at 0.08 ± 0.05 g% (upper limit: 0.18 g%). The upper normal limit for Heinz body formation was set at 10 per 1000 erythrocytes or one percent. By these standards, excessive exposure to toxic chemicals had occurred in every plant studied. Among their observations, it was noted that methemoglobin was short-lived in the blood, being quickly reconverted to hemoglobin, and its presence, therefore, was indicative of acute exposure of short duration. Sulfhemoglobin, on the other hand, disappeared much more slowly from the blood and was formed in response to subacute exposure levels below the threshold amount for subjective symptoms of poisoning. Consequently, elevated sulfhemoglobin levels were regarded as good indicators of prolonged exposure to low concentrations of nitroaromatics. Heinz body appearance in the erythrocytes generally seemed to be an early sign of intoxication as well as low-level exposure, although their formation was often a variable response. Increased Heinz body production paralleled the development of toxic anemias accompanied by erythrocyte destruction, and was claimed to precede many of the clinical signs of anemia, thereby making it a sensitive indicator of exposure.

On the basis of their experiences in this study, the authors established criteria for distinguishing mild, moderate, and severe exposure to aromatic nitro and amino compounds.

Mild Exposure:

1. The contamination of air should not exceed the permissible level.

2. The erythrocyte count and hemoglobin level should be normal.
3. The average methemoglobin value should not exceed the upper limit of normal.
4. The average sulfhemoglobin level should not exceed the upper limit of normal.
5. Heinz bodies should be absent.
6. Urinary excretion of p-aminophenol should be less than 0.7 mg/100 ml.

Moderate Exposure:

1. Air contamination should exceed normal limits but be less than the toxic level.
2. The erythrocyte count and hemoglobin level should be normal.
3. The methemoglobin level should exceed the upper limit of normal, but not be higher than 1.4-2.0 g%.
4. The sulfhemoglobin level should exceed the upper limit of normal, but not be higher than 0.35-0.50 g%.
5. The number of Heinz bodies should exceed one percent.

Severe Exposure:

1. Air contamination should exceed the toxic limit.
2. Erythrocyte count and hemoglobin level should be below normal, or anemia should be present.
3. Methemoglobin values should exceed 1.4-2.0 g%.

4. Sulfhemoglobin values should exceed 0.35-0.50 g%.

5. The number of Heinz bodies should exceed one percent.

It has recently been proposed that the determination of unsaturated iron-binding capacity (UIBC) in the blood can be a sensitive early indicator of exposure to aromatic nitro and amino derivatives. Tarpa *et al*. (1972) measured the UIBC in workers of the dye-stuff industry and noted that exposed workers had UIBC values approximately 50% of the control subject average (Table 81). This technique is far more sensitive, though not as specific, as methemoglobin and sulfhemoglobin determinations as a measure of exposure to toxic nitroaromatic derivatives. It seems that UIBC determinations may be very useful as a part of medical surveillance and monitoring programs in hazardous occupational situations.

Table 81. Mean Values of UIBC in Workers Exposed to Nitro- and Amino-Aromatic Derivatives and in Control (Tarpa *et al*., 1972)

Lot	No. pers.	Sex M	Sex F	UIBC ($X \pm S$)	Statistical Significance
Exposed to poisons	39	31	8	131 ± 35	$p<0.001$
Control	28	18	10	246 ± 34	

a. Trinitrotoluene

By far the greatest number of poisoning cases related directly to nitroaromatic chemical exposure has been among workers involved with the production and handling of trinitrotoluene (TNT). The most serious toxic effects from TNT absorption are liver damage, manifested by jaundice which leads to acute yellow atrophy, and aplastic anemia (a persistent form of anemia, generally unresponsive to therapy). Other major clinical symptoms often include dermatitis, cyanosis, gastrointestinal disturbance, methemoglobinemia, and sulfhemoglobinemia.

A review of the early literature by Von Oettingen (1941) described numerous studies on the incidence of adverse reactions to TNT among industrial workers. He noted that over a 20 month period during World War I, a single plant in the United States reported 7,000 cases of TNT poisoning with 105 fatalities; and another 7 1/2 month period produced 17,000 cases and 475 deaths, some of which were probably due to mixtures of toxic chemicals. Unprotected TNT production workers are known to be very susceptible to intoxication, because TNT dust is readily absorbed through the skin, especially when it is wet with perspiration.

Jaffe _et_ _al_. (1973) have prepared an extensive literature evaluation of TNT toxicity in which they noted that changes in the blood are noted first with both acute and chronic exposures. Damage to the blood may include reduction in the hemoglobin level and red cell count associated with polychromasia (variation in hemoglobin content of the erythrocytes), poikilocytosis (abnormally formed erythrocytes), anisocytosis (the presence of nucleated erythrocytes), reticulocytosis (abnormal increase in reticulocyte number), and eosinophilia (abnormal increase in eosinophil number). Increases are also observed in leukocyte and lymphocyte counts.

In a study of industrial workers by Soboleva (1969), observations were made of cataracts, cholecystitis (inflammation of the gall bladder), hematological changes, neurasthenia (nervous exhaustion), polyneuritis (inflammation of many nerves at once), and hypotonic neurocirculatory dystonia (loss of muscle tone). Gastrointestinal disorders resulting from TNT poisoning were observed by Faerman (1957); they included hyperacidity in those exposed for less than 10 years, and hypoacidity in those with exposures from 10 to 30 years. Makienko and Karamanov (1973) examined the oral cavities of TNT workers and found characteristic carious and non-carious tooth injury and periodontal and oral cavity mucous membrane diseases. Hassman (1971) noted that alcohol ingestion affects the metabolism of TNT, causing reddening of the face and cyanosis of the lips. He described the first symptoms of poisoning as being toxic gastritis, reduced trypsin activity in the pancreatic secretion, increased glomerular filtration, and hypo- or hypermenorrhea in women.

The early effects of exposure to TNT were described by Stewart _et al._ (1944) from a study involving 62 student volunteers working in a filling factory. The major changes noted were in the blood picture, in which over 80 percent of the students were affected. Hemoglobin was decreased by 10-15%, and a mild anemia was observed. Increased levels of reticulocytes became quite pronounced after the worker had left contact with TNT (Table 82, Figure 55). This was probably a compensatory phenomenon indicative of a hematologic repair process which had been inhibited by the TNT.

Table 82. Last Reticulocyte Count at Factory Compared with Value on Return to Oxford Approximately 48 Hours Later (Stewart *et al.*, 1944)

Group	Reticulocytes Average number per c.mm. blood (X 10^4)		
	Before exposure	Last count at factory	Two to four days after cessation of contact
44 Females (I)	3.8	7.5	9.3
10 Males (II)	3.6	8.1	12.6
8 Males (III)	4.3	15.0	27.1

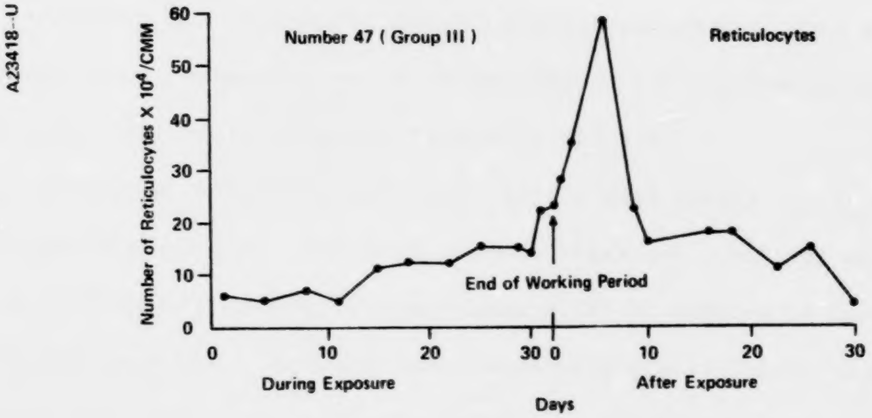

Figure 55. Total Reticulocyte Count During and After TNT Exposure (Stewart *et al.*, 1944)

Clearly, the early effects of TNT exposure involve hemolysis of red blood cells and interference with hematopoiesis. In addition, an erythroblastic reaction in the bone marrow indicates further attempts to compensate for increased destruction of red blood cells. It was found, however, that only after the subject was removed from TNT exposure could the marrow exert full repair activity by producing increased reticulocytes (immature red blood cells). This apparent depression of bone marrow function may account for the cases of severe aplastic anemia which have frequently been observed among TNT workers.

An occupational study conducted by McConnell and Flinn (1946) reported on the cases of 22 fatalities which occurred in government-owned ordnance plants during the period from 1941 to 1945. Of the 22 deaths reported, eight died of toxic hepatitis; 13, of aplastic anemia; and 1 died from a combination of both. While the main route of absorption was through the skin, the lungs and gastrointestinal tract were also considered to be important. The authors pointed out that only a third of those who died were exposed to excessive air concentrations of TNT, and that individual differences such as nutritional state and other illness could alter susceptibility. The influence of age on susceptibility was reflected by the fact that hepatitis occurred more frequently among the younger age group (average age - 30 years) and aplastic anemia among the older age group (average age - 45 years). The median period of exposure to TNT for the hepatitis group was 63 days, as compared to 216 days for the aplastic anemia cases. Diagnosis of TNT poisoning in the early stages was said to be difficult, and clinical signs were often not evident until severe damage had occurred. Among those with toxic hepatitis, the signs of acute toxemia, including deep jaundice and stupor, developed rapidly and were followed shortly by death (median time - 34 days). In the anemia cases, a rapid reduction in erythrocyte count and hemoglobin level signaled severe bone marrow damage, and death shortly ensued (median time - 40 days). Pathological examination of the liver in hepatitis victims revealed degeneration and great reduction in size and weight, with advanced atrophy in the involved areas accompanied by destruction of all parenchymal cells. The pathology of aplastic anemia appeared to be independent of hepatitis and was characterized by multiple petechiae (spots caused by hemorrhage) and diffuse hemorrhage of all organs, including skin and membranes.

Several reports have been made concerning the development of cataracts following occupational exposure to TNT (Soboleva, 1969, Logan *et al.*, 1970). Zakharova and Manoilova (1971) studied the cases of 360 persons exposed to small doses of TNT for a period of at least five years. They found that 45.3 percent of the group developed a single cataract which appeared as the only sign of TNT poisoning.

A case of peripheral neuropathy (disturbance of the peripheral nervous system) and vasculitis (inflammation of the vascular system) in a person sensitive to dynamite (nitroglycerine and TNT) has been reported by Jacob and Maroun (1969). They described the salient neurological symptoms which can accompany sensitivity to organic nitrates, including severe headache due to vasodilation, behavioral disturbance, muscle weakness, and the precipitation of acute violent mental disturbance upon the ingestion of alcohol. Among persons with a strong sensitivity to organic nitrates, contact with clothing, or even shaking the hand of a person who was exposed to these chemicals, could produce clinical symptoms.

In a study conducted in 1952 at Lone Star Ordnance Plant in Texas (Goodwin, 1972), TNT dust and fume concentrations were found in most cases to exceed the established threshold limit value of 1.5 mg/m^3 of air, and ranged as high as 9.5 mg/m^3. Results of this study were used to justify the installation of modern processing equipment, and a reexamination of the situation since 1952 demonstrated that no fatalities or cases of permanent liver damage had occurred. Pre-employment examinations, routine testing for liver impairment, and transferral of persons with sensitivity to TNT are also credited with reducing the incidence of TNT poisoning.

A report has recently been abstracted from the foreign literature concerning the effects of chronic poisoning by nitro-containing toluene compounds among 130 persons (Makotchenko, 1974). The major finding was that a severe disturbance of adrenal cortex function had taken place. This apparent loss of adrenal cortical hormone activity was manifested by such symptoms as polyneuritis, gastric secretory disorders and loss of muscular strength and energy.

b. Tetryl

Another nitroaromatic munitions compound of toxicological significance, which has been produced in large quantities during wartime, is tetryl (N-methyl-N-nitro-2,4,6-trinitroaniline). An account has been presented by Hardy and Maloof (1950) of the cases of munitions factory workers during the period from 1941 to 1945 who became ill or died from tetryl exposure. Yellowish discoloration of the skin and hair affected nearly all workers, and tetryl-induced dermatitis accounted for a high turnover rate in many high-exposure jobs. Those who became seriously ill from tetryl intoxication usually displayed asthma-like attacks of wheezing and violent coughing, accompanied by anorexia (loss of appetite), loss of weight, and degenerative changes of the liver. It was frequently noted that users of alcoholic beverages were much more susceptible to tetryl poisoning. While as many as 34 percent of exposed workers were reported to develop local effects such as dermatitis and irritation of the upper respiratory tract mucous membranes, many investigators noted pronounced systemic toxicity as well. Removal of the patient from the workplace containing tetryl did not always lead to remission of symptoms, and chronic debilitation may persist for years, eventually resulting in death.

Qualitatively, the clinical picture of tetryl intoxication is very similar to that of TNT. The insidious course of events resulting from chronic exposure generally leads to irreversible damage of the liver and other organs, producing permanent disability or death. Clearly, the need for measures to control worker exposure is crucial in the case of munitions compounds.

c. Dinitrobenzene

Dinitrobenzene is generally regarded as the most potent methemoglobin-forming agent of all the nitroaromatics. This characteristic property is beneficial in the sense that intense cyanosis will occur with only slight exposure and thereby serve as a warning of toxic absorption before severe tissue damage can take place. This, unfortunately, is not the case with many nitroaromatic compounds such as TNT, tetryl, or the nitrophenol derivatives. It should be noted, however, that a review of the early literature (Von Oettingen, 1941) showed that 30 fatalities due to dinitrobenzene were recorded, 20 from acute exposures and 10 from delayed effects. The case reports indicated a characteristic acute or subacute atrophy of the liver.

Industrial dinitrobenzene poisoning was a common occurrence during both World Wars, when the chemical was manufactured as a constituent of the explosive roburite (Von Oettingen, 1941). Several cases of poisoning by m-dinitrobenzene were observed by Rejsek (1947), who noted that acute symptoms appeared first as headache, pressure on the chest, general malaise, nausea, and vomiting. Cyanosis soon became manifest and, in severe cases, liver function was impaired, or atrophy developed. The case of a man was reported who had been employed as a munitions worker handling m-dinitrobenzene and after six months suddenly became cyanotic and was sent home. Four weeks after his symptoms had disappeared, the man drank a glass of beer and within three hours became violently ill with nausea, vomiting, headache, and blue coloration. He was placed in a hospital and more than one week later, when all symptoms had disappeared, the patient was given a pint of beer (2% alcohol content). Within two hours, the man became intensely cyanotic and shortly thereafter developed an extreme episode of vomiting which persisted all day. One week later, the patient discovered that while sunbathing for an hour his lips became cyanotic and he developed a severe headache. Several other patients were subsequently seen who had been exposed to m-dinitrobenzene and similarly developed a serious relapse of acute symptoms upon exposure to sunlight or ingestion of alcohol. It was remarkable to note that, even six weeks after the disappearance of toxic symptoms, a complete relapse could occur.

Rejsek (1947) made the observation that the course of poisoning could be affected by the patient's diet and genetic make-up.

Beritic (1956), as well, pointed out that patients display a marked difference in their response to dinitrobenzene, although they may have worked under identical conditions. He presented the cases of two women aged 22 and 25 years who were employed in the manufacture of dinitrobenzene under very similar working conditions. Both women developed classical symptoms of poisoning including headache, fatigue, nausea, and cyanosis. Their clinical pictures, however, were quite different, with one woman developing methemoglobinemia, moderate anemia, enlargement of the liver, and no Heinz bodies; the other woman displayed anemia with Heinz bodies and no evidence of liver damage. It is by no means clear why individuals vary in their response to nitroaromatic exposure, even though it has been a common observation for many years. The explanation most probably involves a complex interaction of factors which apparently includes genetic make-up, nutritional state, body fat stores, and general state of health governing the detoxification mechanisms of the body.

d. Nitrochlorobenzene

The effects of exposure to nitrochlorobenzene are similar in many respects to dinitrobenzene and nitrobenzene poisoning. Cases of occupational nitrochlorobenzene poisoning first began to appear at the beginning of the 20th century. Reports clearly described the symptoms of nausea, vomiting, cyanosis, shortness of breath on exertion, and mild anemia.

The most recent report of professional intoxication was made by Saitá and Moreo (1958), who presented the case of a 25 year old worker accidentally poisoned by inhalation of ortho- and para-nitrochlorobenzene vapors. The symptoms which he displayed were primarily related to toxic effects on the blood. These included cyanosis, methemoglobinemia, Heinz body formation, persistent sulfhemoglobinemia, and hemolytic anemia. Figure 56 details the progression of the characteristic nitroaromatic-induced hematologic changes in this case and illustrates the increases in reticulocytes and protoporphyrins which are known to occur during the reparative stages of acute hemolytic anemia.

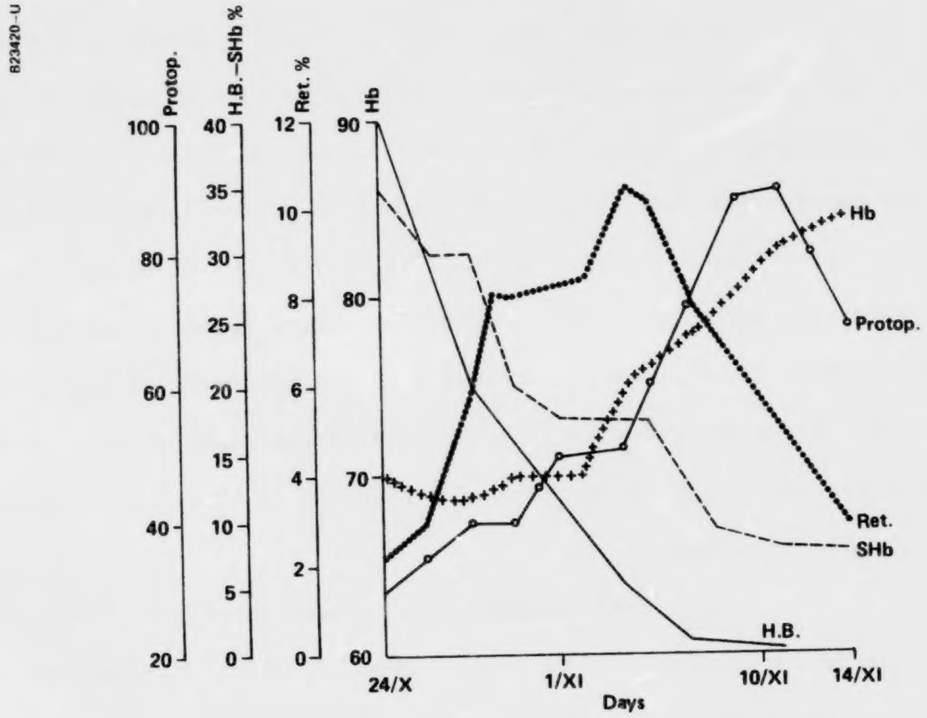

Figure 56. Hematologic Effects From Nitrochlorobenzene Poisoning* (Saita and Moreo, 1958)
* Symbols: Protop. = protoporphyrins; H.B. = Heinz bodies; SHb = sulfhemoglobin; Ret. = reticulocytes; Hb = hemoglobin.

e. 1-Chloro-2,4-dinitrobenzene (DNCB)

The action of DNCB as a potent skin sensitizer is well documented (see Section III-B-4-b), although few cases of occupational exposure have been encountered. A report of four cases of contact dermatitis directly attributable to DNCB exposure has been made by Adams *et al*. (1971). Four persons employed as air-conditioning repairmen became sensitized to DNCB through its use as an algicide in coolant water.

In one case, the patient developed a severe blistering dermatitis of the left arm shortly after spilling a large quantity of an algicide containing 22 percent DNCB on his skin and clothing. The man had previously experienced many episodes of dermatitis following incidents where he had been splattered with the algicide. Another worker developed a severe dermatitis of the right face, neck, right arm, chest, abdomen, and both thighs after an accident in which he was sprayed with the concentrated algicide. Two weeks later, slight contact with water containing the algicide at a concentration of one part per million produced a pruritic (itching) blistering dermatitis of the right hand.

The authors commented that, even though the last previous report of occupational exposure to DNCB occurred in 1928, the compound is nevertheless one of the strongest primary skin irritants known and should be handled with extreme caution. Exposure to DNCB, even in minute concentrations, will almost certainly lead to allergic sensitization. Reactions from the slightest further contact can range from a few pruritic papules or papulovesicles to a widespread exfoliative dermatitis.

Other Skin Sensitizers (see Section III-B-4-b)

Several additional incidents of skin irritation and allergic contact sensitization in man have been reported involving nitroaromatic derivatives.

In one case, a young man working with poultry feed developed a chronic eczema of both hands which improved only during holidays away from work (Bleumink and Nater, 1973). Patch testing with various feed additive substances revealed that a positive allergic reaction was elicited only by 3,5-dinitrotoluamide (Dinitolmide), an anti-coccidial chicken feed additive.

A rare case of contact dermatitis from carbon paper was reported by Calnan and Connor (1972). A woman developed seborrheic dermatitis of the nose, eyebrows, forehead, ears, hands, and fingers after handling a special carbon paper containing nigrosine. Nigrosine is a complex mixture containing either nitrobenzene, nitrophenol, or nitrocresols. Patch testing of the individual components of the carbon paper demonstrated that nigrosine alone was responsible for the skin reaction.

In a controlled human study, Finnegan et al. (1958) tested 50 subjects for sensitivity to pentachloronitrobenzene (PCNB). Thirteen individuals developed positive skin irritation reactions from exposure to PCNB, when patches were applied after an initial sensitizing application two weeks earlier. Four of the persons developed delayed hypersensitivity reactions ranging from eight hours to several days after removal of the PCNB-containing patch from the skin.

f. 2,4-Dinitrophenol

The early history of occupational dinitrophenol poisoning was mainly related to its use in the manufacture of explosives during World War I. The toxic manifestations of dinitrophenol exposure, as reviewed by Horner (1942), included subacute symptoms such as gastrointestinal disturbances (anorexia, nausea, vomiting, colic diarrhea), loss of weight, night sweating, weakness, headache, and dizziness. Acute poisoning resulted in the sudden onset of pallor, burning thirst, profuse sweating, agitation, dyspnea (difficult breathing), and a moderate elevation of temperature. Although prompt removal from the workplace usually brought relief, the exposed alcoholic or person with renal or hepatic disease might die within a few hours. The adverse effects in humans caused by dinitrophenol can be closely linked to its characteristic ability to uncouple oxidative phosphorylation (see Section III-B-4-c).

A review of the early literature by Von Oettingen (1941) revealed that 27 cases of fatal occupational dinitrophenol poisoning had been reported for the years 1914 to 1916; 17 of which occurred in weighing and melting operations, 8 in extraction, and one each in the warming and finishing operations. During the year 1916 to 1917, 31 fatalities were recorded in France and five more in the following 12 months.

Gisclard and Woodward (1946) reported two cases of fatal exposure in workers involved with the manufacture of picric acid from dinitrophenol. The men handled open barrels into which was dumped dinitrophenol, which generated considerable dust and fumes. In both cases, symptoms of fever, profuse sweating, and restlessness became evident after a few months of exposure.

The men were returned to their jobs after palliative treatment and rest, whereupon one worker collapsed and died within four hours after admission to the hospital. One week later, the second man died in a similar fashion. Laboratory investigation of their internal organs revealed a high degree of dinitrophenol absorption.

Several reports of toxic exposure have been summarized by Saita (1949) involving workmen who impregnated wooden posts with a parasiticide powder of dinitrophenol. These cases produced hematologic alterations including hemolytic anemia in one worker, and neutropenia (decreased number of neutrophilic leukocytes) and eosinophilia (increased number of eosinophils) in others.

g. 4,6-Dinitro-*ortho*-cresol (DNOC)

The methyl derivative of dinitrophenol, DNOC, has been widely used as a herbicide and anti-parasite, and has accounted for numerous cases of poisoning and death among agricultural workers. The major hazard to workers occurs during the mixing and application processes, when DNOC is sprayed on cereal crops and orchard trees. The microscopic droplets, or dust, which are formed by spray nozzles create a mist which is easily inhaled and has caused many cases of poisoning.

The qualitative physiologic actions of DNOC in man are very similar to those produced by dinitrophenol. Clinical symptoms which are observed in acute poisoning cases (DiBosco, 1970) include fatigue, intense thirst, profuse sweating causing dehydration, fever, heart failure, dyspnea, nausea, vomiting, and abdominal pain. The skin becomes flushed and often dyed yellow where exposure is greatest (hands, face, feet, knees), and the basal metabolism rate can rise to as high as 400 percent of normal. Additional symptoms can be glucosuria (high glucose levels in the urine), hyperglycemia resulting from the mobilization of carbohydrate reserves, and disturbances of the cardiac rhythm. Actions on the nervous system may cause spasms (convulsive and vocal), delirium, and coma. Death will often occur by respiratory failure resulting from pulmonary edema. Subacute intoxication may become manifest as loss of weight, headache, fatigue, anorexia, increased metabolic rate, dyspnea, and abdominal pain. The characteristic yellow staining of the skin, hair, and urine by DNOC is increased by repeated subacute exposures. Chronic exposure to DNOC will produce mainly an increase in the metabolic rate, loss of weight, fatigue, anxiety, and degeneration of the liver, kidneys, and heart.

A report summarizing eight fatal cases of DNOC poisoning in Great Britain was presented by Bidstrup and Payne (1951). The authors noted that, in nearly all cases, the early symptoms of poisoning such as excessive sweating, unusual thirst, and loss of weight had been ignored by the patient until he became acutely ill with symptoms of increased respiration, fever, and tachycardia (increased heart rate). These acute symptoms were always followed shortly by coma and death. They observed that among the cases of fatal poisoning by DNOC reported throughout the world, many have occurred during unusually hot weather. The relationship between high environmental temperature and the incidence of poisoning may be partly due to the fact that workers remove protective clothing and thereby expose a greater skin surface for possible exposure. Controlled studies in animals, however, have confirmed an increased susceptibility with higher temperatures (see Section III-D).

Bidstrup and Payne also reported a personal communication on three cases of severe intoxication among men engaged in DNOC manufacture. One patient who had worked with DNOC powder, pouring it from kegs into a grinding mill, was employed only 17 days before becoming severely ill. His skin was stained yellow within a few days of beginning work, but he remained on the job until two days before his admission to the hospital. His most striking symptom was profuse sweating which was described by the notation that "the suprasternal notch filled with sweat as one stood and watched."

Two cases of polyneuritis, a condition known to occur with other nitroaromatics, were observed by Stott (1956) in workers exposed to DNOC. Both men were employed in servicing the spray equipment of aircraft used to spray a 20 percent solution of DNOC in oil. It was determined that the major route of DNOC absorption had occurred through the skin. The men presented symptoms of a pins-and-needles sensation on the hands and fingers, accompanied by partial loss of sensation to pin pricks on the hands and feet of one man. The author postulated that a high local concentration of DNOC in the skin due to prolonged contact with the oil solution might exert a local tissue effect before more general symptoms could occur. This hypothesis seems reasonable in light of the finding that localized symptoms and yellow staining occurred at the areas of highest exposure, i.e., hands and feet.

h. Other Nitrophenol Derivatives

Studies were conducted in Washington State from 1956 to 1959 on the health hazards associated with the use of the triethanolamine and isopropanolamine salts of 2-sec-butyl-4,6-dinitrophenol (dinoseb) as a weed-control spray and the sodium salt of DNOC (Na-DNOC) as a blossom-thinning spray on tree fruits (Wolfe et al., 1961). Dermal exposure of spray-equipment operators was determined by exposing absorbent cellulose pads and items of cotton clothing on various parts of their bodies. Hands were rinsed with water in a polyethylene bag to measure skin contamination. Results of the dermal exposure studies are presented in Table 83. Individual variations were noted among workers due to clothing (short-sleeved shirts, open collar, no hat or protective gloves) and also where workers had accidentally spilled concentrated material on themselves. The difference between careful and careless workmen was illustrated by comparing the hand exposure to dinoseb where neither man wore gloves. One worker had an exposure of 95.5 mg per hour, whereas the more careful man had an exposure of 29.6 mg per hour. The value of wearing protective gloves was determined by noting an exposure of 22.4 mg per hour when gloves were worn, and 91.1 mg per hour when they were not.

Determination of respiratory exposure revealed an average inhalation potential of 0.12 mg per hour for dinoseb and 0.03 mg per hour for Na-DNOC. The difference in the two values was probably due to the fact that dinoseb was usually applied at the average rate of five pounds per acre, while Na-DNOC was applied at about 1.3 pounds per acre.

A calculated estimate of the total potential daily exposure for dinoseb was about 4.6 percent of the toxic level, and for Na-DNOC was about 0.9 percent of the toxic level. These figures compare to 43.2 percent of the toxic level for a sprayman using parathion under similar conditions.

A recent report by DiBosco (1970) revealed that 2,298 cases of poisoning among agricultural workers in Italy were reported during the six-year period from 1964 to 1969. Of these cases, four were attributed to

Table 83. Dermal Exposure of Spraymen to DINOSEB and Na-DNOC (Wolfe et al., 1961)

			DINOSEB			Na-DNOC		
Body part	Pad Location or Area Rinsed	Body Area (Sq. Ft.)	No. Pads or Rinses Analyzed	Toxicant Recovery (Mg/Sq. Ft/Hr)	Calculated Exposure (Mg/Body Part/Hr.)	No. Pads Analyzed	Toxicant Recovery (Mg/Sq. Ft/Hr)	Calculated Exposure (Mg/Body Part/Hr.)
Face	Shoulders	0.70	62	1.6	1.1	62	7.0	4.9
Front of neck; "V" of chest	"V" of chest	0.16	33	3.4	0.5	32	2.3	0.4
Back of neck	Back of neck	0.12	32	1.0	0.1	33	5.6	0.7
Forearms	Forearms	1.30	66	7.5	9.7	63	8.5	11.0
	Hands (DNOSBP) Forearms (NA-DNOC)	0.87	48	--*	77.3	63	8.5	7.4
Total	--	3.15	241	--	88.7	190	--	24.4
Less hands	--	2.28	193	--	11.4	190	--	17.0
Thighs	Thighs	3.75	38	31.7	118.9	64	8.1	30.4
Legs	Legs	2.50	30	4.9	12.2	30	3.7	9.2
Above ankles	Above ankles	--	27	1.5	--	17	0.3	--
Total	--	--	95	--	131.1	111	--	39.6

*Bag rinse measurement for hand area.

dinitrophenol derivatives, and one of these resulted in death. Two of the four incidents resulted from exposure to 2-sec-butyl-4,6-dinitrophenyl-3-methylcrotonate (Binapacryl). The classical signs of dinitrophenol derivative poisoning were observed, most notably headache, nausea, dyspnea, and profuse sweating. The third poisoning case was caused by 2-capryl-4,6-dinitrophenyl crotonate (Karathane), which caused an acute allergic dermatitis accompanied by difficult respiration, thirst, and fever. The case which resulted in death involved a man who had mixed an antiparasitic solution of 50 percent DNOC in water. He became ill within 10 hours with vomiting, profuse sweating, muscular tremors, and cerebral spasms. A progressive myocardial infarction and pulmonary failure led to his death. Upon autopsy it was revealed that the man had extensive degenerative lesions of the heart, liver, kidneys and small intestine, which were probably due in part to previous exposures to dinitrophenol compounds.

A generalized clinical picture of poisoning by dinitrophenol derivatives was described by the above authors. Beginning with the uncoupling of oxidative phosphorylation, metabolic processes are intensified, and energy is produced and dissipated in the form of heat. An increase is seen in the passive phase of protein metabolism, with massive mobilization of glucose and fat reserves. Increased frequency of respiration and elevated oxygen consumption are noted, along with the development of acidosis, which may cause tachycardia. If death does not ensue quickly, degeneration of the liver, kidneys, and heart usually takes place. Accumulation of dinitrophenol derivatives, particularly DNOC, was shown to occur in man, which is in contrast to animal models where cumulative effects were not conclusively demonstrated (see Section III-D).

i. Nitroanilines

Of the three isomers of nitroaniline, para-nitroaniline is regarded as the most toxic. Symptoms of p-nitroaniline poisoning are consistent with the known effects of nitrobenzene derivatives in general. These include primarily headache, restricted breathing, nausea, vomiting, and intense cyanosis with violet coloration of the ears, lips, nose, tongue, fingers, and toes. Methemoglobinemia is the main pathologic finding, which usually subsides spontaneously within a short time after removal from exposure.

A report of an incident of acute p-nitroaniline poisoning on a cargo ship was made by Anderson (1946). Seven dock laborers became ill after sweeping up the contents of several kegs of p-nitroaniline which had been broken in the ship's hold. One of the men became unconscious after the day's work; he was admitted to the hospital and found to have enlargement of both the liver and spleen, probably due to a previous malarial hepatitis and spleenitis. He was conscious by the following morning but had developed toxic jaundice with fever, tachycardia, albuminuria (albumin in the urine) and hematuria (blood in the urine). The patient eventually died 50 hours after his first exposure to the p-nitroaniline. The diminished liver and spleen function, due to previous illness, probably led to the development of severe jaundice and death because he was unable to metabolize the poison.

2. Non-Occupational Exposures

The non-occupational exposure of humans to nitroaromatic compounds has produced several important illustrations of their varied and potentially lethal action. Incidents of fatal poisoning and severe intoxication have resulted from both accidental and suicidal ingestion, as well as by administration as a drug in the case of dinitrophenol and DNOC. In the past, many individuals were exposed to commercial products containing potentially harmful concentrations of nitroaromatic ingredients. These cases have pointed out the necessity for manufacturers to carefully consider not only the intended use of a product but also the consequences of exposure resulting from misuse or accidental ingestion. Failure to do so has led to considerable human suffering as well as criminal charges against commercial producers.

a. 2,4-Dinitrophenol and Derivatives

One of the most striking examples of a highly specific toxic effect due to chemical exposure was seen when an outbreak of cataracts in young women occurred in an epidemic fashion during the mid-1930's. The etiology of these incidents clearly indicated a common cause, the administration of dinitrophenol as an anti-obesity agent. A comprehensive review by Horner (1942) pointed out that dinitrophenol was received with overwhelming popularity as a drug, in spite of warnings about harmful side-effects caused by disruption of the metabolic rate. In one study, dinitrophenol treatment was successful in achieving weight loss without dietary restriction in 89.4 percent of those taking the substance at an average daily dose of 300 mg. It was estimated that during the fifteen months following its introduction 100,000 persons were treated with dinitrophenol. In addition, DNOC was also used for weight reduction, being considered three to five times as potent as dinitrophenol.

Toxic reactions to these weight-loss regimens soon began to appear in the literature. Cutaneous lesions were noted in 8 to 23 percent of the patients, as well as various gastrointestinal symptoms, agranulocytosis of the bone marrow, neuritis, cardiovascular complications, and hepatic and renal damage. Nine deaths were reported from dinitrophenol administration and one from DNOC. A narrative account by Parascandola (1974) described the turbulent history and public concern over dinitrophenol in the United States. Controversy was aroused by dramatic articles such as the one appearing in Newsweek in 1933, entitled, "Diet and Die with Excess Alpha Dinitrophenol." This story warned the public about the dangers of taking dinitrophenol by presenting the case history of a physician who had been "literally cooked to death" by an overdose of the drug, which produced extreme hyperthermia (see Section III-B-4-c).

Reports of cataract development attributable to dinitrophenol therapy began to appear in 1935, and, during the next two months, seven more cases were reported. The total number of persons affected with cataracts was estimated at more than 164, before these incidents finally subsided during 1936-1937, when the drug was withdrawn from use. Horner summarized the characteristic features of dinitrophenol-induced cataracts and concluded that, 1) Occurrence was in young women of an age group not normally prone to cataract development, 2) The lesions were bilateral and appeared after weight reduction treatment with dinitro compounds, 3) An interval of months or years may elapse between the last dose of drug given and the onset of symptoms, 4) The lenticular changes occurred with striking similarity, 5) The changes progressed rapidly until vision was obscured, 6) Treatment was not effective in halting their development, and 7) Surgical removal of the cataract was uniformly successful in restoring sight. The mode of action of dinitrophenol in producing cataracts could not be clearly established, and investigators were hampered by the fact that experimental cataracts could not be produced in laboratory animals (see Section III-D).

Acute exposure to dinitrophenol derivatives has produced fatalities in several non-occupational incidents. A case of accidental fatal poisoning by ingestion of a weed-killer containing alkanolamine salts of 2-sec-Butyl-4,6-dinitrophenol (dinoseb) reported by Cann and Verhulst (1960), illustrates the importance of proper labelling and storage of hazardous nitro-aromatic compounds. In this case, a man had ingested and then ejected from his mouth a small quantity of the liquid weed-killer, which he thought was grape juice. His death ensued within 24 hours, and postmortem examination revealed parenchymatous degeneration of the renal tubular epithelium.

Ingestion of concentrated solutions of dinitrophenol have been shown to produce even more dramatic effects, as described by Swamy (1960) in reporting a suicide case. His account was of a man who, after drinking a solution of dinitrophenol with the intention of committing suicide, soon developed a burning sensation in the stomach and vomited yellowish matter. He was taken to the hospital in a state of intense shock and incoherence with feeble pulse and rapid respiration. Death occurred within five to six hours of ingesting the solution. Physical examination revealed that the tongue and mouth were eroded, pupils dilated, conjunctiva congested, and the patient cyanotic with profuse sweating. On postmortem examination additional erosion was found of the tissues of the esophagus and mucous membrane of the stomach.

A recent case of fatal exposure has been reported by Buchinskii (1974) involving dinitro-ortho-cresol (DNOC). An ointment containing DNOC was applied to the skin of a four year old child which caused death accompanied by tachycardia, convulsions, and mucosal hemorrhage.

b. Nitrobenzene

The actions of nitrobenzene on the body can be quite varied, but predominantly include toxic effects on the hematologic system, such as methemoglobinemia and anoxemia due to a low level of normal blood hemoglobin. Respiratory changes occur as the result of direct action on the respiratory center of the brain, and from generalized cerebral anoxemia. Deprivation of oxygen to the brain also leads to CNS reactions including coma, paralysis, involuntary movements, headache, and behavioral changes. The lungs, liver, kidneys, and gastrointestinal system have also been shown to be damaged in certain instances of nitrobenzene poisoning.

Accidental exposure to nitrobenzene has occurred in many persons due to its past commercial use in common substances such as shoe polish and dyes, inks, and even perfumes. Numerous deaths have also resulted when women took nitrobenzene in order to induce abortion (Von Oettingen, 1941). Contact with nitrobenzene has resulted in poisoning of babies wearing diapers stamped with a laundry ink containing the substance. The largest number of related cases of accidental nitrobenzene poisoning, as summarized by Stifel (1919), occurred when 17 persons developed severe cyanosis, headache, malaise, and vertigo after wearing freshly dyed shoes.

An unusual incident of poisoning occurred among five infants who suddenly became cyanotic after being breast-fed by their mothers (Dollinger, 1949). The women had each eaten a piece of cake flavored with an artificial almond substance, presumably containing nitrobenzene, that had subsequently passed into their milk and poisoned the infants.

A case report by Wirtschafter and Wolpaw (1944) described how a drunken man ingested 15 ml of laundry marker ink containing nitrobenzene and became acutely ill. He was brought to the hospital with marked cyanosis of the lips, nails, and ears; and his teeth, tongue, and mucous membranes were stained dark blue. After four days, his color returned to normal, with his pulse and respiration improving in three days. After 14 days, the patient was sent home in good condition.

The authors pointed out that a considerable variation in individual susceptibility has been evident from the past literature on nitrobenzene poisoning. In some cases, as little as one gram of the substance may be fatal, while in others a dose ranging from 3 to 100 grams may be followed by recovery. For example, when nitrobenzene was used as an abortifacient by 16 women in doses of 15 to 100 grams, seven of them died and only one abortion resulted.

Twenty-one infants in Egypt were poisoned by nitrobenzene (Zeitoun, 1959); two cases resulted in death. They had been rubbed with a supposed bitter almond oil, later found to contain 2-10 percent nitrobenzene. The children all showed moderate to intense cyanosis which had developed between four hours and four days after application of the false bitter almond oil. In the cases where death occurred, it was preceded by intense cyanosis, shock, vomiting, difficult respiration, and bronchopneumonia.

Suicidal ingestion of nitrobenzene has accounted for several incidents of serious poisoning. The case of a 24 year old woman was presented by Parkes and Neill (1953). She drank approximately 12 ml of nitrobenzene contained in a bee mixture, in order to commit suicide. She became ill within an hour, with vomiting and dizziness. Intense cyanosis developed and persisted for a week, along with evidence of methemoglobinemia and amino-aciduria (abnormal excretion of amino acids in the urine). She recovered fully in four weeks, however, with no evidence of permanent tissue damage.

More recently, a case has been presented of a 19 year old girl who survived a suicidal ingestion of about 50 ml of nitrobenzene (Myslak _et al_., 1971). Within 30 minutes after the exposure she was hospitalized in an unconscious state with marked cyanosis of the face, ears, palms, feet, and lips. The peripheral blood level of methemoglobin was 82 percent. Prompt medical treatment consisting of gastric lavage, oxygen inhalation, blood transfusion, and drug therapy was probably responsible for saving her life. The clinical course of hematologic effects and excretion of nitrobenzene metabolites is presented in Figures 57, 58, and 59. The patient regained consciousness within two hours, but cyanosis and poor health persisted for ten days along with enlargement of the liver, vomiting, and severe headache. Transient tissue damage to the bone marrow, heart muscle, and liver were noted due to severe hypoxia and general toxemia, but recovery was rapid and complete.

c. Nitroaniline

An unusual outbreak of severe poisoning was recorded by several investigators resulting from the ingestion of wax crayons (Rieders and Brieger, 1953; Brieger, 1949; Jones and Brieger, 1947). The crayons were known to contain a dye, para red, considered to be harmless and shown to be non-toxic when administered to rats, dogs, and cats (Brieger _et al_., 1948). A careful analysis of the crayons, however, revealed that unreacted _p_-nitroaniline, an intermediate in the synthesis of the dye, was present in a number of the pigment and red crayon samples. The symptoms of methemoglobinemia following the ingestion of red wax crayons were, therefore, apparently due to contamination by p-nitroaniline. This situation illustrated the need for careful analysis of pigments prior to their use in products with a high potential for human exposure.

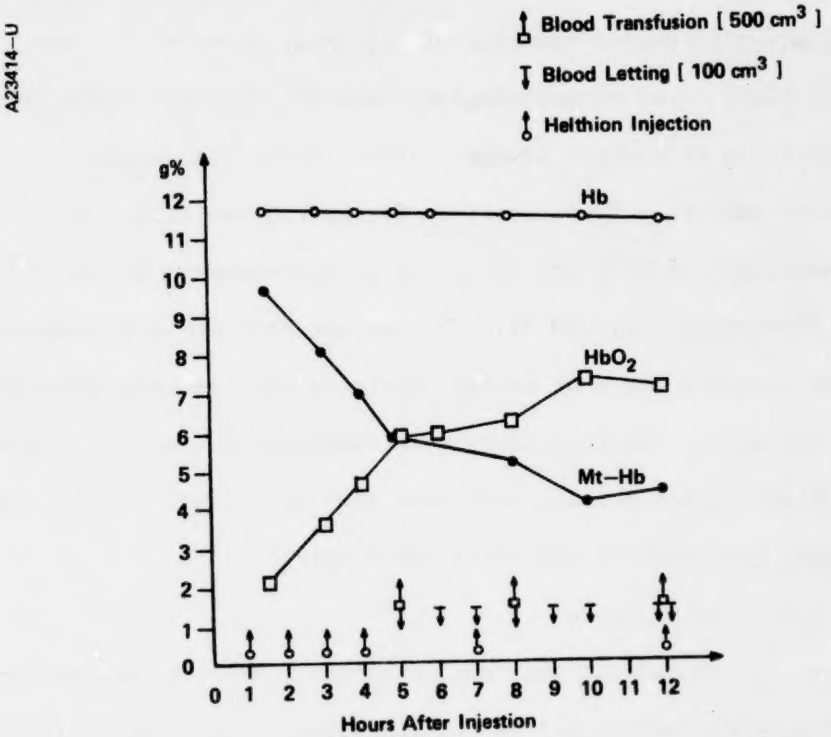

Figure 57. The Level of Methemoglobin and Hemoglobin in Blood During the First 12 Hours of Treatment
(Myślak et al., 1971)

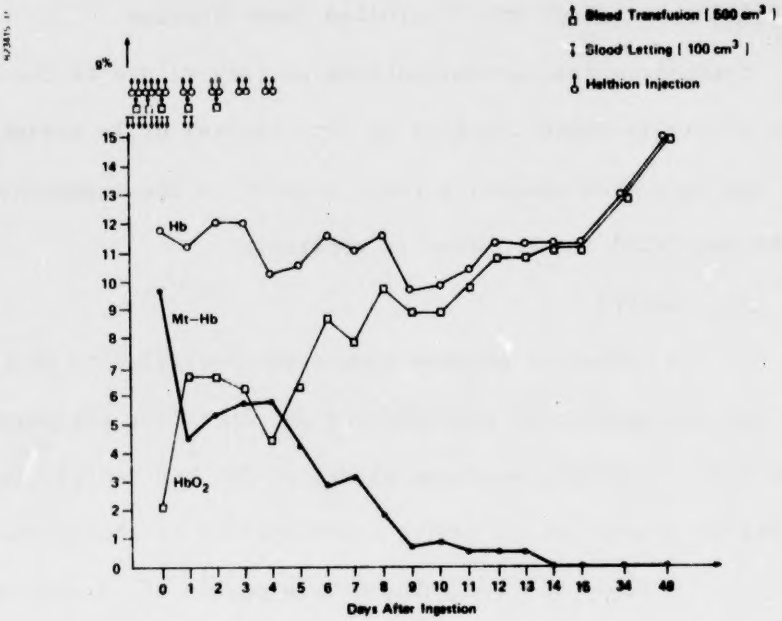

Figure 58. The Level of Methemoglobin and Hemoglobin During the Entire Treatment Period
(Myślak *et al.*, 1971)

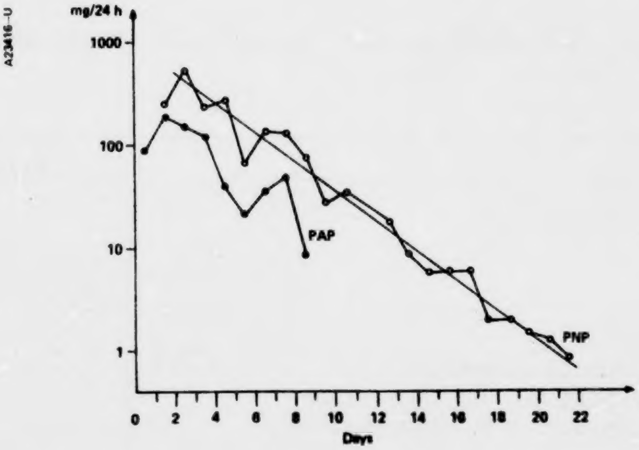

Figure 59. The Excretion Rate (mg/day) of Nitrobenzene Metabolites *p*-Nitrophenol (PNP) and *p*-Aminophenol (PAP) During the Entire Period of Observation
(Myślak *et al.*, 1971)

3. Epidemiological and Controlled Human Studies

Epidemiological investigations as they relate to the nitroaromatic compounds have generally taken the form of experimental or intervention studies. Major concern has been with measuring toxic effects in the industrial environment, where the potential for exposure is greatest.

a. Tetryl

A number of studies have been undertaken to define the extent of occurrence and susceptibility to poisoning by the common nitroaromatic munitions compounds. Emphasis has been placed on TNT and tetryl, because of the large number of documented poisoning cases related to their handling.

A study was conducted over a period of 20 months among 203 workers who were employed in the manufacture of tetryl (Bonenti, 1956). The most frequent symptoms of intoxication among these persons are summarized in Table 84.

Table 84. Incidence of Disturbances of Occupational Origin Among 203 Tetryl Workers (Bonenti, 1956)

Disturbance	Percent frequency
Pruritis (itching) of the face	33%
Frontal headache	25%
Epistaxis	18%
Diarrhea	9%
Restlessness, insomnia	8%
Anorexia, nausea, labored digestion	7%
Other (erythema, dermatitis, cholecystitis, duodenal ulcer, anxiety, rhinopharyngitis	15%

The age of tetryl workers was not found to have an influence on the incidence of disturbance. The number of intoxication episodes could be reduced by limiting the work shift to three days with 15-20 day rest periods in between. Workers who displayed allergic manifestations, or disturbances of the liver or digestive system were removed from the work place due to the high risk of their becoming seriously injured.

In a series of clinical and laboratory investigations conducted by Parmeggiani et al. (1956), over 200 workers who handled tetryl were examined and followed up for two years. The results demonstrated a wide variation among responses to tetryl exposure. These included nosebleed, headache, diarrhea, hyperexcitability, gastro-duodenitis, liver and gallbladder disturbances, itching, and dermatitis. Among 37 cases of dermatitis, only two were found to be the result of allergic responses, the others apparently were due to primary skin irritation. Adoption of measures for the prevention of overexposure to tetryl was stressed in their report; these included the reduction of manual handling procedures and the use of special clothing and protective creams to prevent the contact of tetryl dust with bare skin.

b. Trinitrotoluene

A recent investigation of the effect of TNT exposure on the health of munitions factory workers has been made by El Ghawabi et al. (1974). In their study of 35 workers who had been exposed to continuous TNT concentrations of 0.1 to 1.2 mg/m^3 in air, more than half the group had developed some type of symptom (Table 85). It can be seen from Table 85 that the symptoms displayed were characteristic of those resulting from a local irritant effect rather than a systemic poisoning. Significant to note is the fact that no cases of dermatitis, cataracts, cyanosis, toxic jaundice, or anemia had been discovered, even though these conditions had been reported many times in the past (see Section III-C-1).

Table 85. Incidence of Different Symptoms in Exposed Workers Compared to the Control Group (El Ghawabi et al., 1974)

Symptom	Exposed group		Control group	
	Number of workers with symptoms	Percent of total	Number of workers with symptom	Percent of total
Sneezing	21	60%	1	5%
Sore throat	18	51.4%	1	5%
Cough	16	45.7%	4	20%
Stomach-ache	15	42.8%	1	5%
Loss of appetite	10	28.4%	2	10%
Constipation	13	37.14%	2	10%
Flatulence	12	34.28%	3	15%
Headache	4	11.43%	2	10%
Lassitude	2	5.71%	1	5%
Nausea and vomiting	12	32.28%	1	5%

It is postulated that modern standards of industrial hygiene have accounted for the lack of severe TNT intoxication among the workers in this study. In addition, a high-protein diet which was provided to the workers may have afforded some protection, in light of evidence that a high-fat low-protein diet rendered rats more susceptible to TNT and DNT poisoning.

Very recently, a report was made concerning the hemolytic crisis which ensues from the exposure to TNT by persons with an inherited deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) in the red blood cells (Djerassi and Vitany, 1975). The case histories of three men were presented, all of whom held the same job in a TNT factory, were of Iraqi descent, and were G6PD deficient. Upon exposure to TNT, each of the men developed paleness, enlargement of the spleen and liver, and varying degrees of reticulocytosis and increased urobilinogen in the urine. These symptoms are all indicative of severe hemolysis of the red blood cells.

It should be anticipated that a hemolytic crisis may result in persons with erythrocyte enzyme deficiences, when exposed to nitroaromatic compounds other than TNT and tetryl. Pre-employment medical screening of persons intended for high-exposure jobs will be necessary to identify individuals and populations at high risk.

Several additional epidemiological TNT investigations have been abstracted from the foreign literature. These reports have documented the occurrence of a) liver lesions in 20.8% of a group of 79 TNT-exposed workers (Poljak and Peljuskovic, 1969); b) characteristic carious and non-carious tooth damage and disease of the peridontal and oral cavity mucous membranes (Makienko and Karmanov, 1973); c) gastric disorders and impairment of the secretory function of the stomach in 54% of TNT-poisoned patients (Faerman, 1957; and d) occupational cataracts, toxic hepatitis, cholecystitis, peripheral blood changes, neurasthenia, polyneuritis, and hypotonic neurocirculatory dystonia (Soboleva, 1969; Zakharova and Manoilova, 1971; Manoilova, 1972).

c. Dinitrochlorobenzene (DNCB)

Among the epidemiological studies on non-munitions nitroaromatic compounds, an investigation has been conducted among a population of normal Indian subjects to test skin sensitization by DNCB (Malaviya et al., 1973). Patch testing of the skin for allergic sensitization by DNCB is a simple and commonly-employed method for the quantitation of cell-mediated immunity (Catalona et al., 1972 a, b). Normally, it is possible to sensitize 85-95 percent of the persons exposed to a single application of DNCB. Among 50 Indian volunteers, however, a 100 percent rate of sensitization was achieved. In addition, a relatively small dose of DNCB produced unusually severe spontaneous flare reactions at the application site in 76 percent of the subjects. Furthermore, a very severe delayed hypersensitivity developed in 7 of the 50 persons.

These results suggest a higher level of immunoglobulins in the Indian subjects, and is consistent with the theory that a high prevalence of infectious disease in developing countries can lead to an overactive immune system. The implication of this study is clearly to point out the hazard of hyperreactivity to allergic sensitization by nitroaromatic chemicals among populations with a high level of cell-mediated immunity.

d. Nitrobenzene

A controlled human study, to investigate the effects of low nitrobenzene concentrations in air on several functions of the nervous system, revealed its biological significance as an air pollutant (Andreyeshcheva, 1971). A determination of olfactory sensation to nitrobenzene by 29 volunteers, aged 17 to 35 years, established that remarkably low concentrations of nitrobenzene can be detected by smell in the air (Table 86).

Further studies demonstrated that the smell of subthreshold concentrations of nitrobenzene could progressively decrease the sensitivity of the visual system to light (Figure 60).

Table 86. Results of Determination of Olfactory Sensation Threshold of Nitrobenzene (Andreyeshcheva, 1971)

Number of Subjects	Nitrobenzene Concentration, mg/m^3	
	Threshold	Subthreshold
4	0.0182	0.0169
2	0.023	0.018
7	0.027	0.020
8	0.031	0.024
4	0.037	0.028
1	0.045	0.032
1	0.057	0.011
1	0.070	0.055
1	0.091	0.069

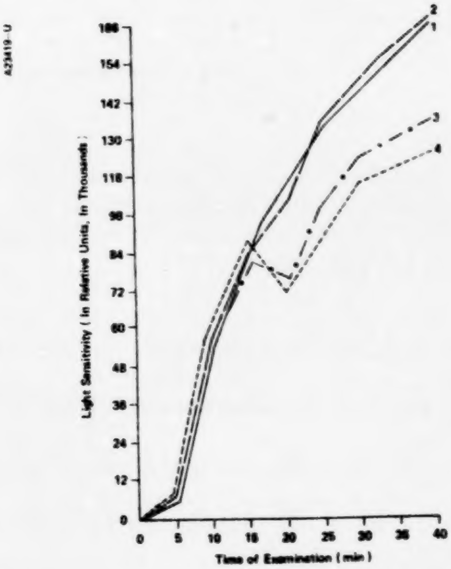

Figure 60. Change in the Light Sensitivity of the Eye During Inhalation of Nitrobenzene in Subject S
1 - pure air; 2 - concentration, 0.0118 mg/m^3;
3 - 0.0157 mg/m^3; 4 - 0.0169 mg/m^3. (Andreyeshcheva, 1971)

Electroencephalographic measurements were also made to determine the effect of nitrobenzene on reflex electrical activity of the brain. In six healthy persons, aged 20 to 35 years, inhalation of nitrobenzene for six minutes at levels below the threshold concentration for smell caused a disruption in the amplitude of intrinsic rhythm potentials of the brain (Figure 61).

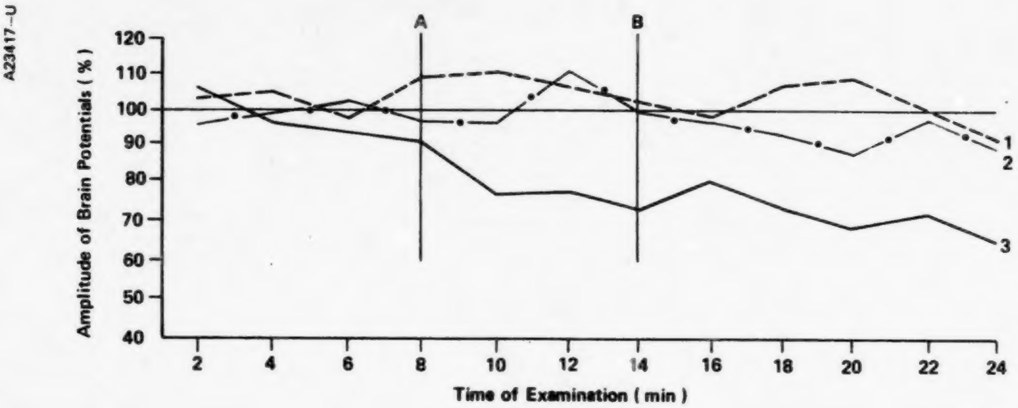

Figure 61. Changes in the Amplitude of Reinforced Intrinsic Potentials of the Brain in Subject L (Andreyeshcheva, 1971)
(1 - pure air; 2 - concentration, 0.008 mg/m^3; 3 - 0.0129 mg/m^3; AP - period of gas inhalation)

The results of this study on nitrobenzene effects indicate that all persons do not exhibit physiological responses at the same levels of exposure. Furthermore, the actual biological consequences of low-level exposure may be sufficiently subtle to avoid detection by conventional means and yet pose a serious threat to health. Linch (1974) has commented, as well, that certain individuals display a profound susceptibility or predisposition to the cyanogenic effects of the nitroaromatic compounds. In reviewing 187 cases of cyanosis, he found that 30 (21%) of the 143 employees involved had contributed 74 (40%) of the cases. Moreover, eight persons, considered to be chronic repeaters, had accounted for 30 cases and were removed from areas of potential exposure.

e. Dinitro-ortho-cresol (DNOC)

The effects of poisoning by DNOC have been studied by oral administration of the compound to five human volunteers (Harvey *et al.*, 1951) (see Section III-B-1-a). The subjects were given 75 mg of pure DNOC daily for five days, examinations were made of the blood and urine, and various physiological responses were tested. These examinations failed to show the presence of Heinz bodies or changes in the reticulocyte count, body weight, pulse rate, respiratory rate, or blood pressure at the dosage level employed (equivalent to 0.92 - 1.27 mg/kg body weight). A yellow coloration of the sclera was evident, however, in all subjects on the fourth day of the experiment. Additional symptoms including headache, lassitude, and general malaise developed in two subjects, which corresponded to the highest blood levels of DNOC achieved in each case. Blood levels of DNOC in all subjects reached the 20 μg/g level after three to five days. Temporary rises above that level were associated with symptoms of poisoning.

In this study, the doses employed were necessarily low and no attempt was made to produce toxic symptoms. The data presented from this investigation demonstrated that DNOC gradually accumulates in the body when ingested at 24-hour intervals and is excreted slowly. Measurable blood levels of DNOC persisted for more than three weeks after the treatment period ended. These results established that DNOC can act as a cumulative poison in man, and imply that other related dinitrophenolic derivatives may very well possess the same cumulative properties.

D. Toxicity - Birds and Mammals

1. Acute Animal Toxicity

Acute toxicity studies using animal model systems usually involve exposures so massive that they bear no practical relationship to expected human environmental exposures. An analysis of the data as summarized in Tables 103-107 (pp. 351-374) is very important, however, in evaluating the potential dangers of nitroaromatic compounds, for at least three specific reasons. One is that it is a means to identify compounds of such extremely low toxicity that single dose exposures may be inconsequential (although chronic exposure hazards may still be high). Secondly, a determination can be made of the possible consequences of high exposures by misuse or accident, with maximum permissible levels for short-term exposure being set accordingly. Finally, compounds with unusual acute toxic properties or extreme toxic potency may be identified and submitted for more extensive chronic studies at realistic subacute dose levels. It is important to note here that acute toxicity studies are of no value in estimating possible carcinogenic, mutagenic, or teratogenic hazards associated with exposure to a chemical substance.

The symptoms of acute poisoning by the nitroaromatic compounds in animals have been found to parallel their effects on humans. They are, for the most part, directly attributable to: 1) action on the hematologic system, 2) the uncoupling of oxidative phosphorylation, 3) central nervous system damage, or 4) stress to the major organs of foreign compound detoxification, namely, the kidneys and liver. The only highly specific toxic effect noted in animals has been the formation of cataracts by exposure to dinitrophenol derivatives and dichloronitroaniline.

The uncouplers of oxidative phosphorylation are evidently limited to the dinitrophenol, dinitroaniline, and nitrosalicylanilide derivatives. The severe hematologic poisons are among the nitrobenzene series of compounds. Transient or irreversible damage to the kidneys and liver appears to be a universal symptom in cases where nitroaromatic exposure is severe.

a. Dinitrophenol and Derivatives

The course of events in acute poisoning by 2,4-dinitrophenol (DNP), 2-sec-butyl-4,6-dinitrophenol (dinoseb), and 4,6-dinitro-ortho-cresol (DNOC) has been well studied, receiving much attention because of their widespread agricultural use. Characteristic symptoms induced in laboratory animals by these compounds are increased respiratory rate, elevated body temperature, increased heart rate, tremors, and the early onset of rigor mortis, with the skeletal muscles becoming stiff just before the animal dies. Mice, rats, rabbits, and dogs all react quite similarly to single doses of the DNP-derivatives.

The time-course of events in poisoning by DNP, resulting from its continuous intravenous infusion in a dog, was provided by Kaiser (1964). This is presented in Figure 62.

The effect of increasing doses of DNP on body temperature and mortality in rats is seen in Table 87 and Figure 63. These data indicate a definite dose-related response to DNP exposure. Responses to acute administration of DNOC are very much the same as for DNP, although the hyperthermic response may not be seen in all cases.

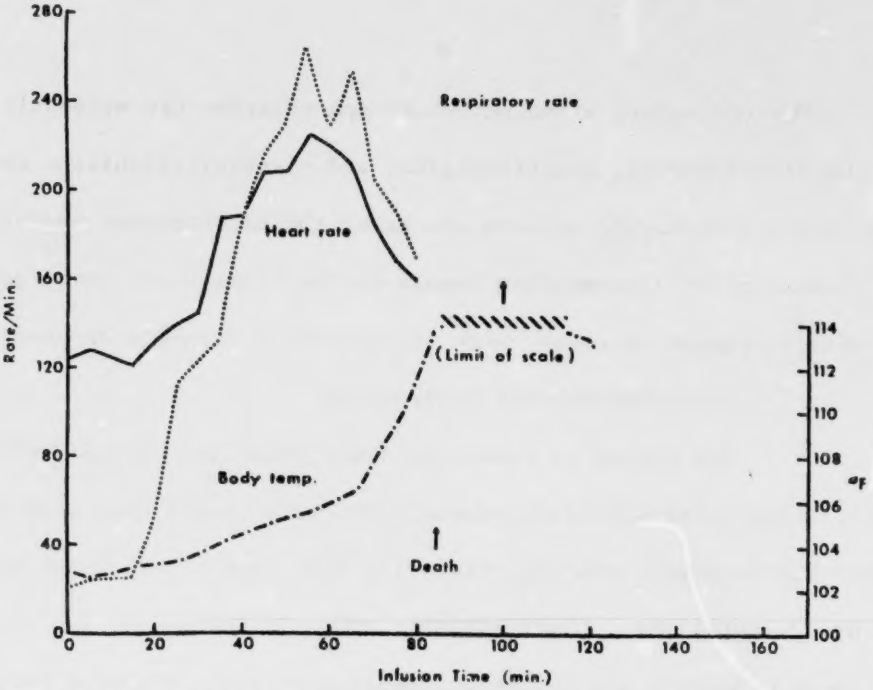

Figure 62. Infusion of Dinitrophenol in Conscious Dog - Rate: 0.4 mg/kg/minute, right external jugular vein (Kaiser, 1964)

Time (min)	Observation
20	Gums, red
25	Ears, moderately red
30	Skin, warm; tongue, purple red; profuse salivation; emesis, 10-15 ml, greenish yellow
35	Nose, warm
40	Tongue extended; emesis, 20 ml, greenish yellow
50	Ears, bright red; emesis, 20 ml, greenish yellow
60	Skin, hot
75	Nose, hot; skin, groin area, yellow
80-83	Dog became stiff and then relaxed
83	Emesis
84-85	Convulsions, death; dose, 36 mg/kg
85-86	All four limbs rigid
60-85	Temperature rose approximately 0.5°F per minute. Temperatures above 113°F are only estimates. Guard on instrument stopped the pointer at an estimated temperature of 113.6°F.

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Table 87. Effect of Logarithmically Increasing Intraperitoneal Doses of 2,4-DNP on Rectal Temperature and Lethality in Rats (Gatz and Jones, 1970)

Group	Number of Animals	2,4-DNP, mg/kg	Δt_{max}*	Percent Mortality	Average Lethal Time, Minutes
1	6	16	1.8 ± 0.2	0	---
2	7	20	2.3 ± 0.2	0	---
3	8	25	3.4 ± 0.3	25	94
4	8	31	6.7 ± 0.4	100	77
5	6	39	4.5 ± 0.3	100	12

* All data are expressed as mean values ± S.E.

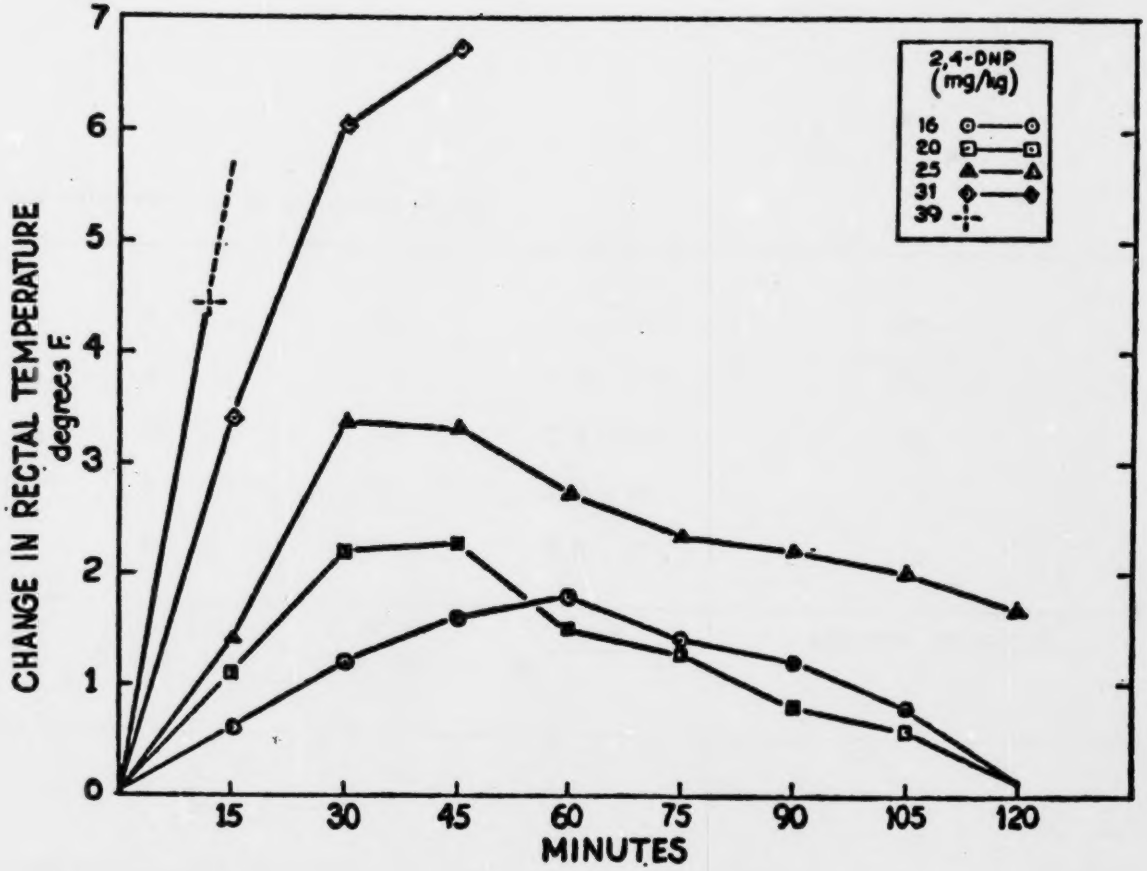

Figure 63. Effect of Logarithmically Increasing Intraperitoneal Doses of 2,4-DNP on Rectal Temperature in Rats (Gatz and Jones, 1970) (Each point on a curve represents the mean of between 6 to 8 determinations of temperature at the specified times.)

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Environmental temperature also affects acute toxicity of the uncoupling compounds such that elevating the ambient temperature increases the mortality from any given dose (Figure 64).

The addition of various groups to the basic DNP nucleus, such as methyl, _sec_-butyl, or cyclohexyl, does not cause drastic alterations in their relative animal toxicities. Addition or rearrangement of nitro groups, on the other hand, may affect toxicity considerably, as shown in Tables 88 and 89.

The presence of a nitro group in a _para_-position to the hydroxyl seems to be correlated with a higher toxicity by DNP and its various derivatives; it also appears to be linked to the production of a hyperthermic response. This characteristic may be due to an increased half-time of elimination from the blood for the _para_-substituted compounds.

In animal experiments, DNP, DNOC, dinoseb, and 2-cyclohexyl-4,6-dinitrophenol did not produce significant skin irritation or primary sensitization in rabbits and guinea pigs (Spencer _et al_., 1948). It was possible, however, to produce death by skin absorption following a single application of either DNP, DNOC, or dinoseb. The cyclohexyl derivative was not toxic by the dermal route, probably due to its poor absorption across the skin (see Section III-B-1).

An analysis of dose-response relationships for the dinitrophenol derivatives has demonstrated a certain degree of unusual variability in the effect of DNP on mortality from a single oral dose as compared to topical exposure (Figure 65). However, DNOC, dinoseb, and 2-cyclohexyl-4,6-dinitrophenol are relatively consistent in conformance to a dose-related increase in mortality (Figures 66-68).

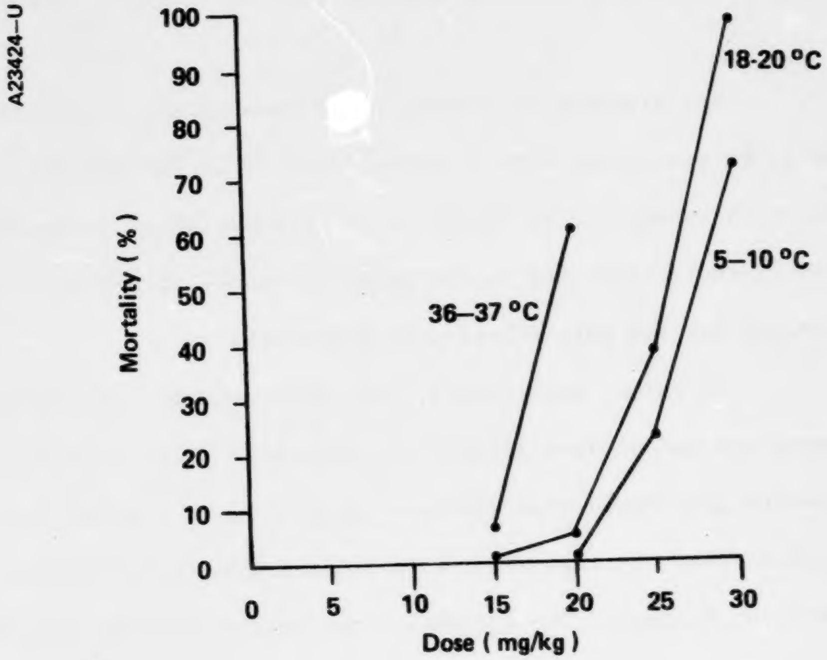


Figure 64. Effect of Environmental Temperature on Mortality in Rats Caused by a Single Dose of Dinitro o-cresol (Parker et al., 1951)

Table 88. Comparison of LD_{50} Values of Dinitrophenols (LD_{50} for 2,4-DNP = 1) (Harvey, 1959)

Dinitrophenol	Rats	Mice
2,3-	5.4	5.5
2,4-	1.0	1.0
2,5-	4.3	7.6
2,6-	1.1	1.3
3,4-	2.8	3.1
3,5-	1.3	1.4

Table 89. Comparison of LD_{50} Values in Rats for Dinitro- and Trinitrocresol (Harvey, 1953)

Substance[a]	LD50 mg/kg
Dinitro-*o*-cresol	24.2
Dinitro-*p*-cresol	24.8
Trinitro-*m*-cresol	168.0

[a] Given as 0.5 percent solutions in 0.5 percent NaCl, 0.5 percent $NaHCO_3$ by intraperitoneal injection

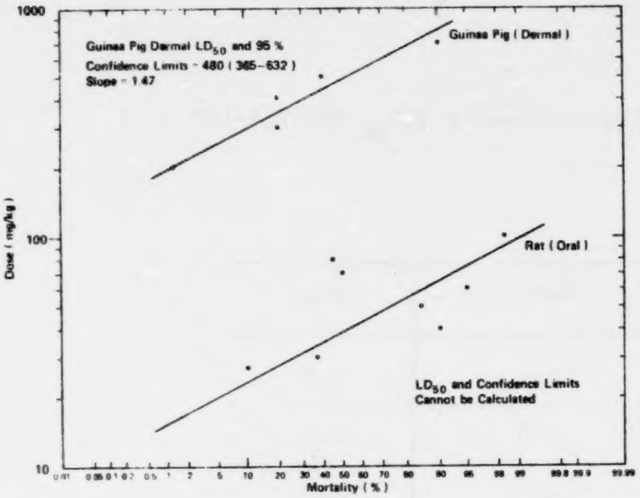


Figure 65. Mortality From a Single Dose of DNP by Oral and Dermal Administration* (Spencer et al., 1948)

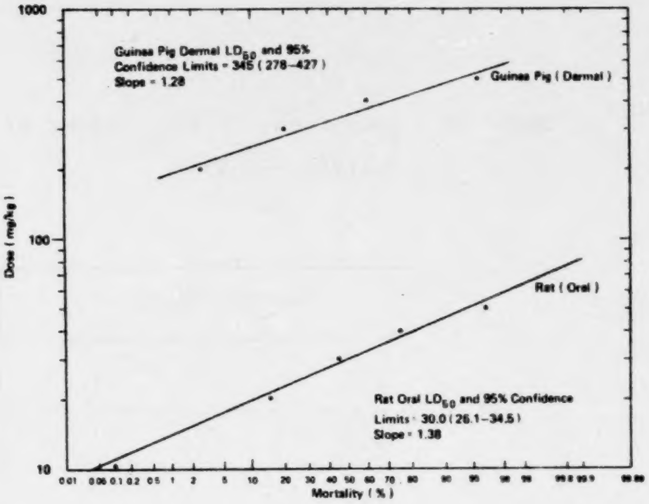


Figure 66. Mortality From a Single Dose of DNOC by Oral and Dermal Administration* (Spencer et al., 1948)

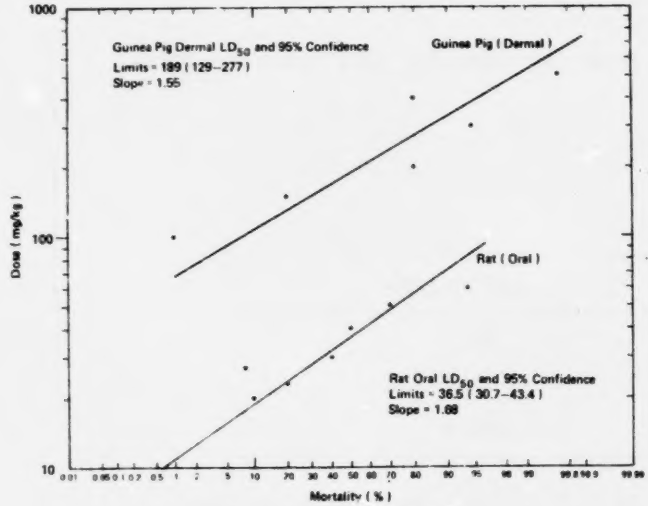


Figure 67. Mortality From a Single Dose of Dinoseb by Oral and Dermal Administration* (Spencer et al., 1948)

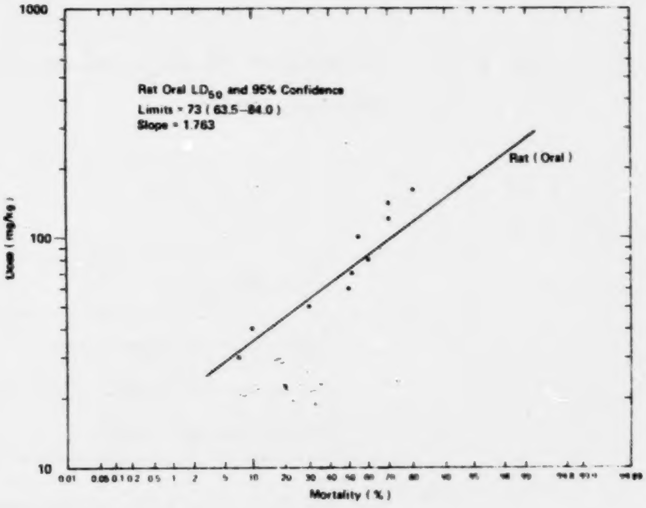


Figure 68. Mortality From a Single Oral Dose of 2-Cyclohexyl-4,6-dinitrophenol in the Rat* (Spencer et al., 1948)

*** Statistical analysis performed by the method of Litchfield and Wilcoxon (1949)**

The above results indicate that, with the exception of 2-cyclohexyl-4,6-dinitrophenol, these compounds produce significant lethal effects over a relatively narrow dosage range. This principle tends to hold true among different mammalian species as well and is illustrated by Figure 69, which depicts the effect on different species of a single oral dose of dinoseb.

Many authors have observed changes in the circulatory system which were attributed to central nervous system stimulation by the dinitrophenols. These effects include both the slowing and raising of the pulse, as well as increased blood pressure. An increase in the respiratory rate has also been a nearly universal observation in poisoned animals. It is not entirely clear whether the effects of dinitrophenol substances on the circulation and respiration are the result of a direct action on the central nervous system or are a secondary response to anoxemia. It has been established, however, that DNP, DNOC, and p-nitrophenol can directly stimulate both the aortic and carotid chemoreceptors of the dog (Shen, 1962). This stimulation produces a marked elevation of respiration, which parallels the hyperthermic action of the three compounds.

(i) Acute Cataract Development

The unusual phenomenon of cataracts of the eyes produced by acute exposure to DNP, DNOC, and their derivatives was first demonstrated in animals almost ten years after the problem was known to exist in humans (Robbins, 1944). Experimental cataracts, first produced in ducks and chickens, differ from DNP-induced human cataracts in that they can be formed by acute exposures and may appear in less than one hour. Furthermore, these lesions will disappear spontaneously in animals within 24 hours.

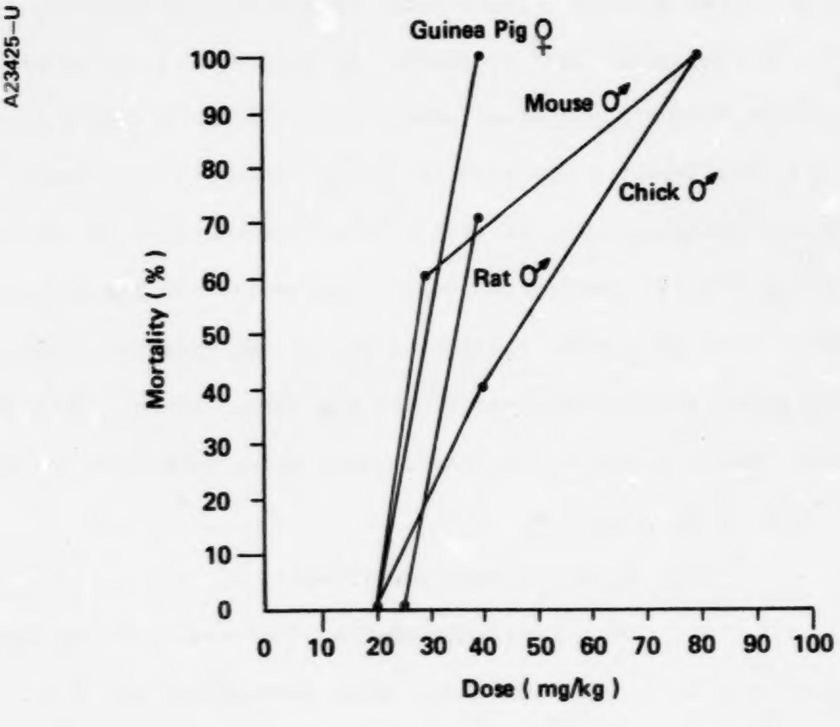


Figure 69. Acute Oral Toxicity of Dinoseb to Various Animal Species (Bough *et al*., 1965)

Buschke (1947) tested 37 compounds chemically related to DNP for their cataractogenic activity by single-dose administration to adult chickens. The results, summarized in Table 90, identified eight compounds which were positive cataract-forming agents.

Table 90. Nitroaromatic Compounds with Cataractogenic Activity in Chickens (Buschke, 1947)

Compound	mmol/kg	Route	Time of Onset (hrs)
2,4-Dinitrophenol	0.06	oral	3½
	0.11	oral	2
	0.11	I.M.	2
	0.22	oral	1
	0.43	oral	1
2,6-Dibromo-4-nitrophenol	0.13	oral	3
	0.27	oral	2
2,4-Dinitroanisole	0.40	oral	5
	0.40	I.M.	4
2,4-Dinitrophenetole	0.38	oral	2
	0.38	I.M.	2
2-Chloro-4,6-dinitrophenol	0.28	oral	4
5-Chloro-2,4-dinitrophenol	0.73	oral	3/4
	0.73	I.M.	3/4
2,6-Dinitrophenol	0.43	oral	5
4,6-Dinitro-o-cresol	0.0125	oral	4-5
	0.02	oral	2
	0.025	oral	2
	0.10	oral	1

In terms of structure-activity correlations, it is important to note that the presence of *ortho*- and *para*- ring substituents is essential to cataract-producing activity. A nitro group placed *para* to the hydroxyl seems to be of great importance. Presence of the phenolic hydroxyl group is a necessity, and any type of substitution for it abolishes activity. The author explained that cataractogenic activity of 2,4-dinitroanisole and 2,4-dinitrophenetole may be due to the biotransformation of the ether to the free phenol in the body.

Table 91 compares the relative cataractogenic potential of various nitroaromatic compounds. The addition of a second ring system to

Table 91. Comparison of Cataract Producing Activities of Various Nitro Compounds in Chickens (2,4-Dinitrophenol = 1) (Buschke, 1947)

4,6-DN-Cresol	4.8
2,4-DN-Phenol	1.0
2,4-DN-Anisole	≥0.5
2,4-DN-Phenetole	≥0.5
2,6-Dibromo-4-Nitro-Phenol	≥0.5
2-Chloro-4,6-DN-Phenol	≥0.22
5-Chloro-2,4-DN-Phenol	<0.16>0.08
2,6-DN-Phenol	0.14
m-DN-Benzene	<0.125 or 0
Mono-Nitro-Phenols	
2-Chloro-4-Nitro-Phenol	
2,4-DN-Naphthol	
2,4-DN-Thymol	
Nitro-Salicylic Acids	
2,4-DN-Chlorobenzene	
2,4-DN-Toluene	
2,4-DN-Mesitylene	
2,4-Dichloro-Phenol	

the molecule abolishes all activity. A fairly close correlation exists between the ability to produce cataracts and the ability to raise the metabolic rate, suggesting a possible relationship between effects on the eye and ability to uncouple oxidative phosphorylation. The cataracts formed in these experiments were all reversible, despite the continued presence of the drug in the diet, and therefore did not precisely resemble the cataracts caused in humans from chronic DNP ingestion (see Section III-C-2-a).

A more recent series of investigations by Gehring and Buerge (1969) established that cataracts could be produced both in ducks and rabbits by acute administration of DNP. Table 92 presents the results of treating ducks by several routes with DNP, and reveals that a definite dose-related response exists for cataract development.

Table 92. Incidence of Cataracts in Ducks Following the Administration of a Single Dose of 2,4-Dinitrophenol (Gehring and Buerge, 1969)

Age (days)	Route of Administration	Dose (mg/kg)	No. with Cataracts per No. Treated	Percent Effect
16-30[a]	Oral	12	0/4	0
		15	0/4	0
		20	3/8	38
		25	3/4	75
		28	3/3	100
		30	4/4	100
16-30[b]	Intraperitoneal	3	3/10	30
		6	6/10	60
		9	10/10	100
		12	5/8	63
		14	9/10	90
		16	9/9	100
75[c]	Intraperitoneal	4	2/5	40
		6	4/5	80
		9	5/5	100
		12	4/4	100

[a] ED_{50} + 0.95 confidence limits equals 21.5 (17.9-25.8) mg/kg

[b] ED_{50} equals 4.7 (3.05-7.24) mg/kg

[c] ED_{50} equals 4.4 (3.12-6.20) mg/kg

With oral administration, cataracts were first seen within one to three hours, while similar opacities were often noted within 30 minutes after intraperitoneal injection.

The production of cataracts by intraocular injection in ducks was very rapid, some appearing in less than 10 minutes. They could be induced by very small doses of DNP ranging from 0.1 to 10.0 micrograms (Table 93). Similarly, the treatment of rabbits by intraperitoneal or intraocular injection, and by *in vitro* incubation of the lens with DNP, could produce rapid cataract formation (Tables 94 and 95).

Table 93. Incidence of Cataracts in Ducklings Following a Single Injection of 2,4-Dinitrophenol Into the Posterior Chamber of the Eye (Gehring and Buerge, 1969)

Dose (μg)[a]	No. with Cataracts per. No. Treated	Percent Effect
10.00	2/2	100.0
5.00	2/2	100.00
2.50	2/2	100.00
1.00	2/2	100.00
0.50	2/2	100.00
0.25	2/4	50.0
0.10	1/4	25.0
Control[b]	0/18	0.0

[a] The indicated dose of 2,4-dinitrophenol was contained in 10 μl of sterile isotonic saline, pH 7.5 ED_{50} equals 0.20 (0.11-0.35) μg.

[b] Controls were obtained by injecting 10 μl of isotonic saline, pH 7.5, into the posterior chamber of the eye contralateral to the eye treated with 2,4-dinitrophenol.

Table 94. Incidence of Cataracts in 90-Day-Old Rabbits Following a Single Injection of 2,4-Dinitrophenol Into the Posterior Chamber of the Eye (Gehring and Buerge, 1969)

Dose (μg)[a]	No. with Cataracts per No. Treated	Percent Effect
50.0	2/2	100.00
10.0	2/2	100.00
5.0	2/4	50.0
2.5	0/2	0.0
Control[b]	0/10	0.0

[a] The indicated dose of 2,4-dinitrophenol was contained in 10 μl of sterile isotonic saline, pH 7.5. ED_{50} equals 5.0 (3.8-6.6) μg.

[b] Controls were obtained by injecting 10 μl of sterile isotonic saline, pH 7.5, into the posterior chamber of the eye contralateral to the eye treated with 2,4-dinitrophenol.

Table 95. Cataract Production in Rabbit Lenses Incubated for 24 Hours at 37° in KEI-4 Media Containing Varying Concentrations of 2,4-Dinitrophenol (Gehring and Buerge, 1969)

Source of Lenses[a]	Concentration of 2,4-Dinitrophenol (M)	No. with Cataracts per. No. Treated
Mature rabbits	1.0×10^{-3}	4/4
	1.0×10^{-4}	4/6
	1.0×10^{-5}	2/6
	1.0×10^{-6}	0/5
	0	2/21
Newborn rabbits	1.0×10^{-4}	6/6
	2.5×10^{-5}	3/3
	1.0×10^{-5}	1/3
	1.0×10^{-6}	0/3
	0	1/15

[a] Mature rabbits were 90-119 days old; newborn rabbits were less than 5 days old.

Table 96 illustrates that increasing age obviously reduced the susceptibility of rabbits to cataract formation. The development with age of mechanisms to rapidly metabolize and excrete DNP before it reaches the lens may very well account for this fact. Further evidence is provided in Table 95, which shows that isolated lenses of mature and newborn rabbits are almost equally vulnerable to cataract formation when incubated in vitro with DNP.

Table 96. The Dose of 2,4-Dinitrophenol Causing a 50% Incidence of Cataracts in Various Age Groups of Rabbits Following Intraperitoneal Administration (Gehring and Buerge, 1969)

Age (days)	ED_{50} (mg/kg)
10 ± 1	6.6 (4.8-9.1)[a]
18 ± 1	9.6 (7.6-12.1)
26 ± 1	14.5 (11.2-18.7)
32 ± 1	18.5 (14.8-23.1)
42 ± 2	23.3 (19.4-27.9)
62 ± 3[b]	32.0 (27.1-37.4)

[a] 0.95 Confidence limits

[b] The ED_{50} value given for 62-day-old rabbits is not statistically valid because the dose necessary to cause cataracts killed some of the rabbits.

An extrapolation of these results to the known cases of DNP-induced cataract development in humans suggests that certain individuals may be rendered more susceptible to the actions of DNP depending on their drug-metabolizing capabilities. The unexpected observation of cataract formation in dogs and pigs by chronic treatment with 2,6-dichloronitroaniline (DCNA) presents further proof of mammalian susceptibility to lenticular damage by certain nitro-substituted phenols (see Section III-D-2). The accidental observation that exposure to sunlight was essential for the cataractogenic action of DCNA implies that the underlying mechanism for this effect may depend upon several variables.

b. Nitrobenzene and Derivatives

Clearly, the acute toxicity of nitrobenzene and its chloro-, methyl-, and amino-substituted derivatives can be most closely associated with hematologic alterations. Almost without exception, acute exposure of animals to these compounds shortly results in the appearance of intracorpuscular Heinz bodies (see Section III-B-4). A sign of severe erythrocyte damage, the appearance of Heinz bodies in the blood signals the development of anemia. Accompanying this reaction is a characteristic compensatory rise in the reticulocyte count. In addition, the nitrobenzene-derived compounds are capable of producing large quantities of methemoglobin and sulfhemoglobin. This feature is in contrast to the apparent lack of hemotoxic effects caused by the dinitrophenol derivatives.

A comparative study of the relative methemoglobin-forming properties of the various nitrobenzene derivatives, when given by intraperitoneal injection to cats, was undertaken by Bredow and Jung (1943). The data presented in Table 97 clearly indicate the potent action of m-dinitrobenzene and 2,4,6-trinitrobenzene in causing hematologic alterations. It is obvious from Table 102 that increasing the methyl-group substituents or decreasing the number of nitro substituents tends to decrease the methemoglobin-forming properties of the compound.

A comparison of dose-related methemoglobin responses to several nitroaromatic chemicals (Table 98) presents strong evidence that the formation of methemoglobin is probably not the primary factor in causing mortality by exposure to these compounds. Note, however, that the oral LD_{50} for nitrobenzene in rats is approximately three-fold less than that for p-nitrotoluene (Table 98), and that methemoglobin formation by these compounds maintains a similar ratio at certain dosages. Therefore, the relative methemoglobin-

Table 97. Comparative Toxicity of Some Nitroaromatic Compounds in Cats (Bredow and Jung, 1943)

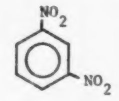
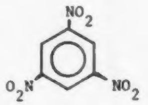
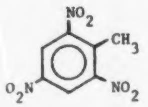
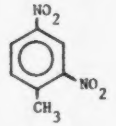
Substance	Formula	Number of Experiments	Amount Methemoglobin / Mole Substance	Time to Maximum Methemoglobin Formation (hours)	Heinz Bodies
m-Dinitrobenzene		13	7.8 (max. 10.3)	10	+ + +
2,4,6-Trinitrobenzene		10	4.8 (max. 7.0)	1	+ + +
2,4,6-Trinitrotoluene		13	1.7	3	+ + +
2,4-Dinitrotoluene		7	1.4	5	0

Table 97. Comparative Toxicity of Some Nitroaromatic Compounds in Cats (Bredow and Jung, 1943) (Cont'd)

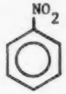
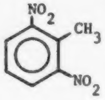
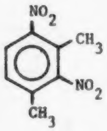
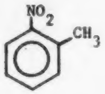
Substance	Formula	Number of Experiments	Amount Methemoglobin / Mole Substance	Time to Maximum Methemoglobin Formation (hours)	Heinz Bodies
Nitrobenzene		16	0.86 (max. 1.5)	6	+ +
2,6-Dinitrotoluene		23	0.55	5	+
4,6-Dinitro-1,3-xylene		8	0.1	8	+
o-Nitrotoluene		6	0.05	8	+

Table 97. Comparative Toxicity of Some Nitroaromatic Compounds in Cats (Bredow and Jung, 1943) (Cont'd)

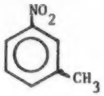
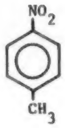
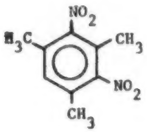
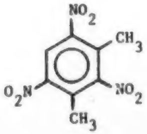
Substance	Formula	Number of Experiments	Amount Methemoglobin / Mole Substance	Time to Maximum Methemoglobin Formation (hours)	Heinz Bodies
m-Nitrotoluene		7	0.04	6	+ +
p-Nitrotoluene		12	trace	--	+ +
2,4-Dinitromesitylene		3	0	--	0
2,4,6-Trinitro-1,3-xylene		2	0	--	0

Table 98. Acute Effects of Several Nitroaromatic Compounds Given by Intraperitoneal Injection to Rats (Magos and Sziza, 1958)

Nitrobenzene			*p*-Nitrobenzaldehyde			*p*-Nitrotoluene		
Dose (mMol/kg)	% Methemoglobin	Time to Death (hrs)	Dose (mMol/kg)	% Methemoglobin	Time to Death (hrs)	Dose (mMol/kg)	% Methemoglobin	Time to Death (hrs)
1.76	44.5	---	1.19	38.6	---	2.04	6.5	---
2.73	33.0	---	1.85	47.9	---	3.06	6.9	---
4.69	34.0	---	2.78	50.0	---	4.53	21.7	
6.79	44.5	24-48	4.10	---	2-3	6.86	23.6	24-48
9.29	58.0	8-24	6.22	---	2-3	10.21	16.0	24-48
13.69	58.0	8-24	9.26	---	2-3	15.30	27.1	24-48

forming properties of the nitrobenzene derivatives may be a good indicator of overall toxic potential.

The members of the nitrobenzene series differ significantly from the nitrophenols in that they do not produce profound metabolic disruption by the uncoupling of oxidative phosphorylation. Damage to the central nervous system, however, is just as severe by the nitrobenzenes as with other nitroaromatic compounds. In chronic studies (see Section III-D-2), nitrobenzene-induced damage to the central nervous system preceded hematologic effects.

c. Trinitrotoluene (TNT)

A noticeable lack of published information concerning the acute toxicity and LD_{50} values for TNT in laboratory animals has been encountered in the preparation of this report. Several lethality determinations were made during the 1920's (Von Oettingen, 1941; Jaffe et al., 1973) which indicated that considerable variation existed in the sensitivity to acute TNT poisoning among the common mammalian species (Table 105). Although extensive comparative studies have not been reported as yet, a number of investigations are now under way to test the acute toxicity of munitions compounds, including TNT and dinitrotoluene (Glennon, 1975).

d. Structure-Activity Relationships

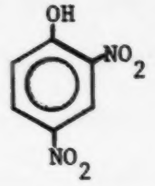
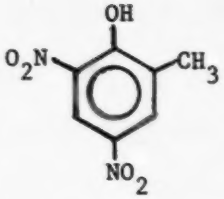
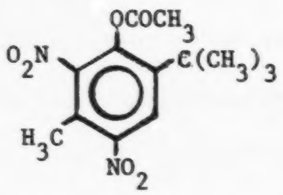
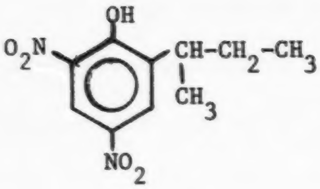
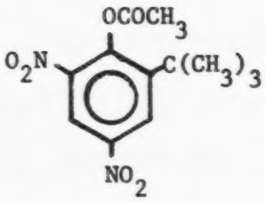
A definitive statement probably cannot be made, based on the currently available toxicity information, regarding the role of specific nitroaromatic molecular structures in producing acute toxic effects; neither has the mode of action of nitroaromatic chemicals at the target organ been established. It is helpful to point out, however, that certain correlations do seem to exist between the toxic potency, on a molar basis, of a nitroaromatic compound and the presence or absence of various ring substituents.

Among the nitroaromatic compounds, the dinitrophenol derivatives possess the greatest degree of acute toxicity, as measured in terms of lethality. Within this group, it is essential to have nitro substituents which are ortho- and para- to the hydroxyl group for greatest toxicity to occur (Table 104). Furthermore, the addition of a second ortho group, such as methyl or butyl, will cause additional activity, while another nitro group or halogen in that position will reduce toxic potency. Toxicity greatly diminishes when either the hydroxyl or para-nitro group is replaced. These relationships are illustrated in Table 99, which presents several nitroaromatic agricultural chemicals in order of decreasing toxicity. The effect of various ring substituents in producing toxicity can be clearly seen as hydroxyl and para-nitro groups are replaced and the number of substitutions on the ring increases.

Among the nitrobenzene derivatives, dinitrobenzenes are more toxic than mononitrobenzenes, although neither group is as toxic as dinitrophenols. Further derivatization of nitrobenzenes into nitroanilines, nitrotoluene, and nitrobenzoic acid will reduce toxicity significantly. In addition, increasing the number of halogen substitutions (e.g., PCNB) and nitro groups (e.g., TNT, tetranitroaniline, trinitrobenzene) in the molecule will also greatly reduce toxicity. Throughout the entire series of nitroaromatic compounds derived from a benzene skeleton, the importance of a para-positioned nitro group for highest toxicity seems to stand out.

Attempts have been made to relate the acute toxicity of the dinitrophenols to their relative potencies as uncouplers of oxidative phosphorylation with some degree of success (Ilivicky and Casida, 1969). A comparison was made of the levels for in vitro uncoupling in mitochondrial preparations and their relation to LD_{50} values (Table 100). These data reveal a fair correlation between increasing uncoupling activity in liver mitochondria and mortality produced in intact mice.

Table 99. Comparative Toxicity of Various Nitroaromatic Structures in the Rat

Structure	Name	Acute Oral Rat LD_{50}[a] (mg/kg)
	2,4-dinitrophenol (DNP)	30[b]
	4,6-dinitro-*o*-cresol (DNOC)	10-50
	2-*tert*-butyl-5-methyl-4,6-dinitrophenyl acetate (medinoterb acetate)	42
	2-*sec*-butyl-4,6-dinitrophenol (dinoseb)	50-60
	2-*tert*-butyl-4,6-dinitrophenyl acetate (dinoterb acetate)	62

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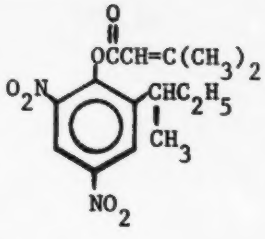
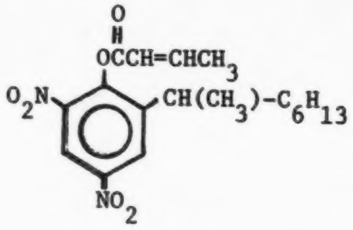
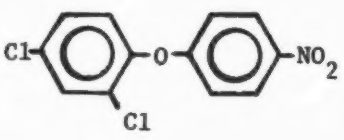
[a] Berg, 1972

[b] Schafer, 1972

Table 99. Comparative Toxicity of Various Nitroaromatic Structures in the Rat (Cont'd)

Structure	Name	Acute Oral LD$_{50}$[a] (mg/kg)
	2-*sec*-butyl-4,6-dinitrophenyl-3-methyl-2-butenoate	161 ± 25
	2,4-dinitro-6-octylphenyl crotonate (dinocap, karathane)	980
	2,4-dichlorophenyl-*p*-nitrophenyl ether (TOK, nitrofen)	2,630

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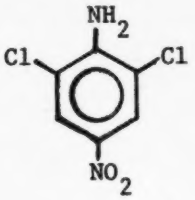
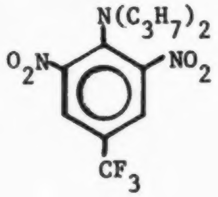
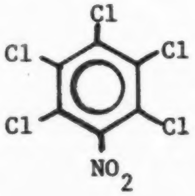
[a] Berg, 1972

[b] Schafer, 1972

Table 99. Comparative Toxicity of Various Nitroaromatic Structures in the Rat (Cont'd)

Structure	Name	Acute Oral LD_{50}[a] (mg/kg)
	2,6-dichloro-4-nitroaniline (botran, dicloran)	>5,000
	2,6-dinitro-N,N-di-*n*-propyl-α,α,α-trifluoro-*p*-toluidine (trifluralin, treflan)	>10,000
	pentachloronitrobenzene (PCNB, quintozene)	>12,000

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[a] Berg, 1972

[b] Schafer, 1972

Table 100. Potency of Various 2,4-Dinitrophenols as Uncouplers of Oxidative Phosphorylation *In Vitro* and Their Toxicity to Mice, Houseflies, and Honey Bees (Ilivicky and Casida, 1969)

2,4-Dinitrophenol Derivative	Minimum Uncoupling Concentration, µ[M], with Mitochondrial Preparations from					LD_{50}, Injected (µmoles/kg)		
	Mouse		Housefly	Honey Bee		Mouse	Housefly	Honey Bee
	Liver	Brain	Thorax	Head	Thorax			
Unsubstituted (DNP)	50	1.0	50	30	50	141	1,630	108
6-Cyclohexyl (DNOCHP)	1.5	1.0	0.2	0.8	1.0	95	131	3.5
6-Methyl (DNOC)	20	20	20	20	30	94	732	18
6-*sec*-Butyl (dinoseb)	1.0	0.5	0.8	0.5	0.8	42	146	11
6-*sec*-Butyl-1-isopropyl carbonate (Dessin)	80	80	100	100	200	383		

When the dinitrophenols were injected into mice, however, a very good correlation was found to exist between uncoupling of brain mitochondria and the severity of overt poisoning symptoms (Table 101). The single exception to this observation was unsubstituted DNP, where severe toxic symptoms were produced without significant uncoupling in brain mitochondria. The suggestion was made that rapid potentiation of the metabolism and elimination

Table 101. Uncoupling and Inhibition of Brain and Liver Mitochondria After Injection of Mice with Various Dinitrophenols (Ilivicky and Casida, 1969)

Compound	Dose (μmoles/kg)	Holding Time (min)	Severity of Symptoms[a]	Magnitude of Uncoupling or Inhibition[b] Brain	Liver
	2,4-Dinitrophenol (DN) uncouplers				
DNP	269	20	2	0	0
6-Cyclohexyl-DNP	38	20	0	0	0
	94	20	2	2	1
Dinoseb	63	20	0	0	0
	100	20	1	1	1
	150	20	2	2	2
Dessin	181	30	1	1	0
	181	60	2	2	0
Dinocap	275	30	1	1	0
	550	30	2	2	2

[a] The severity of the symptoms is graded as follows: 0, not any, same control; 1, mild, survival of animal is probable; 2, severe manifestation, death of animal is expected.

[b] Effects on mitochondria are graded as follows: 0, not any, same as control; 1, partial uncoupling or inhibition; 2, complete uncoupling or inhibition.

(continued on p. 375)

Table 102. Acute Animal Toxicity of Various Nitroaniline Derivatives

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Chloro-4-nitroaniline	Mice	500	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	50	I.V.	Lethal dose	Christensen and Luginbyhl, 1974
	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
4-Chloro-2-nitroaniline	Mice	63	I.V.	LD_{50}	Christensen and Luginbyhl, 1974
	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
4-Chloro-3-nitroaniline	Starling	>100	Oral	LD_{50}	Schafer, 1972
	Blackbird	100	Oral	LD_{50}	Schafer, 1972
2,6-Dichloro-4-Nitroaniline	Rats	>5000	Oral	LD_{50}	Berg, 1972
	Rats	418	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	1500 - 8000	Oral	LD_{50}	Ben-Dyke et al., 1970
	Mallard Duck	>2000	Oral	LD_{50}; regurgitation, ataxia, weakness, wing drop, falling when walking; symptoms may persist for five weeks	Tucker and Crabtree, 1970
2,4-Dinitroaniline	Rats	1800	Oral	LD_{50}; respiration inhibited; uncoupling of oxidative phosphorylation	Vasilenko et al., 1974
	Rats	250	I.P.	Lethal dose	National Academy of Science, 1953

Table 102. Acute Animal Toxicity of Various Nitroaniline Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,4-Dinitro-6-bromoaniline	Rats	4490	Oral	LD_{50}	Christensen and Luginbyhl, 1974
N-Methyl-N,2,4,6-Tetra-nitroaniline	Dog	5000	S.C.	Lethal dose	Christensen and Luginbyhl, 1974
4-(Methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline	Mice and rats	>2000	Oral	LD_{50}	Berg, 1972
	Rabbits	>2000	Dermal	LD_{50}	Berg, 1972
m-Nitroaniline	Rats	900	Oral	LD_{50}; methemoglobinemia and sulfhemoglobinemia at 450 mg/kg	Vasilenko *et al*., 1974
	Rats	535	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	450	Oral	LD_{50}; hematologic changes; severe degeneration of kidneys, liver and spleen	Akahori, 1954
	Mice	308	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rabbits	500	Oral	Methemoglobinemia, Heinz body formation, reticulocytosis, decreased erythrocyte count	Akahori, 1954
	Dogs	70	I.P.	Death within three hours	VonOettingen, 1941

Table 102. Acute Animal Toxicity of Various Nitroaniline Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
o-Nitroaniline	Rats	3564	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	3520	Oral	LD_{50}; hepatotropic effects	Vasilenko *et al.*, 1974
	Mice	308	Oral	LD_{50}	MacEwen and Vernot, 1972
p-Nitroaniline	Rats	3249	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	1410	Oral	LD_{50}; increased methemoglobin and sulfhemoglobin	Vasilenko *et al.*, 1974
	Rats	1500	Oral	LD_{50}; increased erythrocytes, reticulocytes, leukocytes, Heinz bodies, hemoglobin; spasms, lymphopenia	Moskalenko, 1966
	Mice	812	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	250	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
	Guinea Pigs	450	Oral	LD_{50}: increased erythrocytes, reticulocytes, leukocytes, Heinz bodies, hemoglobin; spasms, lymphopenia	Moskalenko, 1966
	Starling	>100	Oral	LD_{50}	Schafer, 1972
	Blackbird	75	Oral	LD_{50}	Schafer, 1972
Tetranitroaniline	Dogs	2500	S.C.	Minimum lethal dose; death within 6 days	VonOettingen, 1941

Table 103. Acute Animal Toxicity of Various Nitrobenzene Derivatives

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
1,2-Dichloro-4,5-dinitrobenzene	Mice	125	i.p.	Lethal dose	Christensen and Luginbyhl, 1974
1,2-Dichloro-5-nitrobenzene	Rats	643	Oral	LD_{50}	Christensen and Luginbyhl, 1974
2,5-Dichloronitrobenzene	Rats	1210	Oral	LD_{50}	" " "
3,4-Dichloronitrobenzene	Rats	50	Oral	No mortality produced	Hanavan, 1975
	Rabbits	200	Dermal	No mortality produced	Hanavan, 1975
_m_-Dinitrobenzene	Rats	50	Oral	No mortality produced	" "
	Dogs	600 mg*	Oral	Minimum lethal dose	VonOettingen, 1941
	Dogs	10	i.v.	Serum iron rose sharply after one day and returned to normal in six days	Cammerer _et al._, 1949
	Dogs	10-20	i.v.	Methemoglobinemia, verdoglobinemia, Heinz bodies, liver damage, cerebral paralysis, convulsions, anemia, increase white cells; LD_{50} was about 10 mg/kg	Kiese, 1949
	Rabbits	400-500 mg*	Oral	Minimum lethal dose	VonOettingen, 1941
	Rabbits	200	Dermal	No mortality produced	Hanavan, 1975
	Blackbird	42	Oral	LD_{50}	Schafer, 1972
	Starling	>100	Oral	LD_{50}	" "
_p_-Dinitrobenzene	Cats	29	Oral	Lethal dose	Christensen and Luginbyhl, 1974

Table 103. Acute Animal Toxicity of Various Nitrobenzene Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
2,4-Dinitrochlorobenzene	Rats	1593	Oral	LD_{50}	Smythe et al., 1962
	Rats	500	Oral	LD_{50}	Edson et al., 1964
	Rabbits	193.6	Dermal	LD_{50}; severe skin and corneal irritation	Smythe et al., 1962
Dinitrotrichlorobenzene	Rats	500	Oral	LD_{50}	Edson et al., 1964
1-Fluoro-2,4-dinitrobenzene	Rats	50	Oral	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	100	s.c.	Lethal dose	" " "
1-Fluoro-4-nitrobenzene	Rats	250	Oral	Lethal dose	" " "
Nitrobenzene	Rats	640	Oral	LD_{50}	" " "
	Rats	664	Oral	LD_{50}	Smythe et al., 1970
	Rats	836	i.p.	100% mortality, death within 24 to 48 hours; 44.5% methemoglobin level	Magos and Sziza, 1958
	Rats	100-200	s.c.	Formation of sulfhemoglobin, nitroxyhemoglobin, methemoglobin, Heinz bodies	Vasilenko and Zvezdai, 1972
	Rats	800	s.c.	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	996	i.p.	100% mortality within 24 hours; central nervous system involvement such as loss of righting reflex within 10 to 15 minutes, coma, shallow respiration, tremor, respiratory arrest	Smith et al., 1967
	Mice	480	s.c.	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	482 mg*	dermal	Prostration, dyspnea; majority died within 24 hours	VonOettingen, 1941
	Mice	50-80 mg/ℓ	inhalation	Toxic level	Pislaru et al., 1962

Table 103. Acute Animal Toxicity of Various Nitrobenzene Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
Nitrobenzene (Cont'd)	Dogs	750-1000	Oral	Minimum lethal dose	VonOettingen, 1941
	Dogs	500-700	Oral	Salivation, unrest, tremors, delirium, increased pulse, staggering gait, clonic and tonic convulsions	" "
	Dogs	750	Oral	Lethal dose	Christensen and Luginbyhl, 1974
	Dogs	150-250	i.v.	Minimum lethal dose	VonOettingen, 1941
	Rabbits	600	Oral	Lethal dose	Christensen and Luginbyhl, 1974
	Rabbits	600	Dermal	Lethal dose	" " "
m-Nitrochlorobenzene	Rats	555	?	LD_{50}	Davydova, 1965
	Mice	390	Oral	LD_{50}	Alishev and Osipov, 1966
	Mice	400	?	LD_{50}	Davydova, 1965
	Rabbits	520	?	LD_{50}	" "
o-Nitrochlorobenzene	Rats	50	Oral	No mortality produced	Hanavan, 1975
	Rats	268	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	135	Oral	LD_{50}	" " "
	Rabbits	200	Dermal	No mortality produced; not corrosive to skin	Hanavan, 1975
p-Nitrochlorobenzene	Rats	812	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	670	Oral	Approximate lethal dose	Hanavan, 1975
	Rats	420	Oral	LD_{50}	Christensen and Luginbyhl, 1974

Table 103. Acute Animal Toxicity of Various Nitrobenzene Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
p-Nitrochlorobenzene (Cont'd)	Rats	500-600	?	Metabolic disturbance of the brain, loss of adrenalin from the adrenals, methemoglobinemia	Frenkel and Gordienka, 1958
	Mice	1414	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	650	Oral	LD_{50}	Alishev and Osipov, 1966
	Rabbits	500	i.p.	Reduced blood pressure and myocardial glycogen level	Labunskii, 1972
	Rabbits	500	s.c.	Severe methemoglobinemia (20% of total hemoglobin), Heinz bodies in almost all erythrocytes within 16 hours; death within 24 hours	Nogawa, 1961
	Rabbits	200	Dermal	No mortality produced	Hanavan, 1975
Pentachloronitrobenzene	Rats	1650	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	1740	Oral	LD_{50}	Borzelleca *et al.*, 1971
	Rabbits	4000	Dermal	No adverse effects were seen during a 14 day observation period when applied to abraded skin	" " "
1,2,4-Trichloro-5-nitrobenzene	Starling	>100	Oral	LD_{50}	Schafer, 1972
	Blackbird	100	Oral	LD_{50}	" "
Trinitrobenzene	Rats	505	Oral	LD_{50}; depression, hyperpnea, gasping, cyanosis, salivation, tachycardia, coma, loss of reflexes, hemorrhagic lungs, discoloration of the blood	Fogleman *et al.*, 1955

* Total dose

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Amino-4-nitrophenol	Mice	1280	Oral	LD_{50}	Akahori, 1954
2-*sec*-Amyl-4,6-dinitrophenol	Mice	4	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
2-Chloro-4,6-dinitrophenol	Rats	500	Oral	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	125-500	I.P.	Approx. LD_{50}	Doull *et al.*, 1962
2-Chloro-4-nitrophenol	Rats	100	Oral	Lethal dose	Christensen and Luginbyhl, 1974
3-Chloro-4-nitrophenol	Mice	125	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
2,4-Dichloro-6-nitrophenol	Rats	100	Oral	Lethal dose	Christensen and Luginbyhl, 1974
2,6-Diiodo-4-nitrophenol	Rats	170	Oral	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
	Rats	105	I.V.	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
	Rats	105	I.P.	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
	Rats	122	S.C.	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,6-Diiodo-4-nitrophenol (Cont'd)	Mice	212	Oral	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
	Mice	88	I.V.	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
	Mice	110	S.C.	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigidity of limbs prior to or immediately after death	Kaiser, 1964
2,6-Dibutyl-4-nitrophenol	Rats	450	Oral	LD_{50} females	Vesselinovitch *et al.*, 1961
	Rats	500	Oral	LD_{50} males	Vesselinovitch *et al.*, 1961
	Rats	260	I.P.	LD_{50} females	Vesselinovitch *et al.*, 1961
	Rats	270	I.P.	LD_{50} males	Vesselinovitch *et al.*, 1961
	Rats	300-600	I.P.	100% mortality; histopathologic changes of the liver, spleen, kidneys, heart, lungs, and lymphoid tissues	Vesselinovitch *et al.*, 1961
	Mice	850	I.P.	LD_{50} females	Vesselinovitch *et al.*, 1961
	Mice	700	I.P.	LD_{50} males	Vesselinovitch *et al.*, 1961
	Guinea Pigs	580	I.P.	LD_{50}	Vesselinovitch *et al.*, 1961
	Guinea Pigs	800	Oral	LD_{50}	Vesselinovitch *et al.*, 1961

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
4,6-Dinitro-2-sec-butylphenol	Rats	40-60	Oral	LD_{50}	Bailey and White, 1965
	Rats	50	Oral	LD_{50}	Ben-Dyke et al., 1970
	Rats	30-60	Oral	LD_{50}	Schafer, 1972
	Rats	60	Oral	100% mortality; largest dose survived by all treated animals was 50 mg/kg	Spencer et al., 1948
	Rats	25-40	Oral	LD_{50}; prostration, rapid respiration, convulsions preceding death, death within 24 hours	Bough et al., 1965
	Rats	50	Oral	LD_{50}	Edson et al., 1964
	Rats	21.4	S.C.	LD_{50}	Harvey, 1952
	Rats	200-600	Dermal	LD_{50}	Edson et al., 1964
	Rats	80-200	Dermal	LD_{50}	Ben-Dyke et al., 1970
	Rats	80	Dermal	LD_{50}	Christensen and Luginbyhl, 1974
	Mice	20-40	Oral	LD_{50}; prostration, rapid respiration, convulsions preceded death, death within 24 hours	Bough et al., 1965
	Mice	10.1	I.P.	LD_{50}	Ilivicky and Casida, 1969
	Mice	100	Dermal	20% mortality; 90% mortality at 500 mg/kg	Bough et al., 1965
	Guinea Pig	20-40	Oral	LD_{50}; prostration, rapid respiration, convulsions preceded death, death within 24 hours	Bough et al., 1965

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
4,6-Dinitro-2-*sec*-butylphenol (Cont'd)	Guinea Pigs	500	Dermal	100% mortality; largest dose survived by all treated animals was 100 mg/kg	Spencer *et al*., 1948
	Starling	7.1	Oral	LD_{50}	Schafer, 1972
4,6-Dinitro-*o*-cresol	Rats	33	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	10-50	Oral	LD_{50}	Berg, 1972
	Rats	25-40	Oral	LD_{50}	Edson *et al*., 1964
	Rats	30	Oral	LD_{50}	Bailey and White, 1965
	Rats	50	Oral	Minimum fatal dose	Corti, 1953
	Rats	25-40	Oral	LD_{50}	Ben-Dyke *et al*., 1970
	Rats	28.5	I.P.	LD_{50}	Lawford *et al*., 1954
	Rats	24.6	S.C.	LD_{50}; death usually occurred within two hours	Parker *et al*., 1951
	Rats	20	S.C.	Respiration rate increased rapidly after 10 to 15 minutes and animals became prostrated, which persisted for one to two hours followed by gradual recovery; where death occurred, it was preceded shortly by muscular rigidity which was complete when the animal died and persisted for some hours after death	Parker *et al*., 1951
	Rats	25.6	S.C.	LD_{50}; LD_{50} range of four DNOC commercial preparations was 26.2 to 27.5 mg/kg	Harvey, 1952
	Rats	200-600	Dermal	LD_{50}	Edson *et al*., 1964

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
4,6-Dinitro-*o*-cresol (Cont'd)	Rats	200-600	Dermal	LD_{50}	Ben-Dyke *et al.*, 1970
	Mice	21	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	24	I.P.	LD_{50}	Lawford *et al.*, 1954
	Mice	24.2	I.P.	LD_{50}; sweating, increased respiration, diminished physical activity, marked rigor mortis	Harvey, 1953
	Mice	18.7	I.P.	LD_{50}	Ilivicky and Casida, 1969
	Mice	24.2	S.C.	LD_{50}; death usually occurred within two hours	Parker *et al.*, 1951
	Mice	47 mg/m^3	Inhalation	50% mortality	Christensen and Luginbyhl, 1974
	Rabbits	10-40	Oral	Neurotoxic syndrome developed within 5 to 15 minutes; decreased erythrocyte and increased leukocyte counts; 45 mg/kg was fatal	Arustamyan, 1973
	Rabbits	23.5	I.P.	LD_{50}	Lawford *et al.*, 1954
	Guinea Pigs	22.5	I.P.	LD_{50}	Lawford *et al.*, 1954
	Guinea Pigs	200	Dermal	Maximum tolerated dose; minimum fatal dose was 500 mg/kg	Corti, 1953
	Mallard Duck	22.7	Oral	LD_{50}; ataxia, wings crossed high over back, tail tremors or shivering, falling when walking, tachypnea, dyspnea, unkempt feathers, tetany with the legs extended posteriorly; symptoms persisted in some survivors for up to two weeks	Tucker and Crabtree, 1970

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,4-Dinitro-6-octyl-phenylcrotonate (dinocap, Karathane)	Rats	980-1190	Oral	LD_{50}	Edson et al., 1964
	Rabbits	>9400	Dermal	LD_{50}	Edson et al., 1964
2,6-Dinitro-p-cresol	Mice	24.8	I.P.	LD_{50}	Harvey, 1953
4,6-Dinitro-o-cyclohexylphenol	Rats	180	Oral	100% mortality; largest dose survived by all treated animals was 30 mg/kg	Spencer et al., 1948
	Rats	250	Oral	Lethal dose	National Academy of Science, 1953
	Mice	100	Oral	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	25.3	I.P.	LD_{50}	Ilivicky and Casida, 1969
	Guinea Pigs	1000	Dermal	No mortality produced	Spencer et al., 1948
2,4-Dinitrophenol	Rats	30	Oral	LD_{50}	Schafer, 1972
	Rats	71	Oral	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964
	Rats	32.7	I.P.	LD_{50}	Lawford et al., 1954
	Rats	35	I.P.	LD_{50} at 18-21° environmental temperature	Harvey, 1959
	Rats	10-20	I.P.	Oxygen consumption increased 17 to 21 percent	Harvey, 1959
	Rats	50	I.P.	100% mortality	Obbink and Dalderup, 1964
	Rats	25	I.P.	25% mortality; average time to death was 94 minutes	Gatz and Jones, 1970

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,4-Dinitrophenol (Cont'd)	Rats	31	I.P.	100% mortality; average time to death was 77 minutes	Gatz and Jones, 1970
	Rats	39	I.P.	100% mortality average time to death was 12 minutes	Gatz and Jones, 1970
	Rats	60	I.P.	LD_{50}; tremors, prostration, increased respiration, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964
	Rats	25	S.C.	LD_{50}; 10 mg/kg caused no deaths while 50 mg/kg produced 100% mortality	Tainter and Cutting, 1933
	Mice	72	Oral	LD_{50}; tremors, prostration, increased respiratory rate, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964
	Mice	52	I.P.	LD_{50}; tremors, prostration, increased respiration, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964
	Mice	25.9	I.P.	LD_{50}	Ilivicky and Casida, 1969
	Mice	26	I.P.	LD_{50}	Lawford *et al*., 1954
	Mice	36	I.P.	LD_{50} at 18-21°C environmental temperature	Harvey, 1959
	Mice	>5	I.P.	100% mortality at 39-41°C environmental temperature	Harvey, 1959
	Mice	56	I.V.	LD_{50}; tremors, prostration, increased respiration, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,4-Dinitrophenol (Cont'd)	Mice	58	S.C.	LD_{50}; tremors, prostration, increased respiration, tonic convulsions, rigor mortis prior to or immediately after death	Kaiser, 1964
	Rabbits	30	S.C.	LD_{50}	Tainter and Cutting, 1933
	Dogs	30	I.P.	Minimum lethal dose	Harvey, 1959
	Dogs	22	S.C.	LD_{50}; no deaths were caused by doses up to 20 mg/kg but 25 mg/kg produced 100% mortality	Tainter and Cutting, 1933
	Dogs	20–30	I.V.	LD_{50}	Tainter and Cutting, 1933
	Dogs	20	I.M.	LD_{50}	Tainter and Cutting, 1933
	Starling	46	Oral	LD_{50}	Schafer, 1972
	Blackbird	13	Oral	LD_{50}	Schafer, 1972
2,4-Dinitro-6-phenylphenol	Mice	31	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
4-Ethyl-2,6-dinitrophenol	Mice	30	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
4-Isopropyl-2,6-dinitrophenol	Mice	31	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
2-(α-Methylbenzyl)-4,6-dinitrophenol	Mice	63	I.P.	Lethal dose	Christensen and Luginbyhl, 1974

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
4-Nitro-*m*-cresol	Mice	500	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
2-Nitro-*p*-cresol	Rats	3360	Oral	LD_{50}	Christensen and Luginbyhl, 1974
m-Nitrophenol	Rats	933	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1414	Oral	LD_{50}	MacEwen and Vernot, 1972
	Dogs	83	I.V.	Lethal dose	Christensen and Luginbyhl, 1974
o-Nitrophenol	Rats	2828	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1297	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	600	I.M.	Lethal dose	Dittmer, 1959
	Rabbits	1700	S.C.	Lethal dose	Dittmer, 1959
	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
	Cats	600	S.C.	Lethal dose	Dittmer, 1959
	Dogs	100	I.V.	Lethal dose	Dittmer, 1959
p-Nitrophenol	Rats	350	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	616	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	97	I.P.	LD_{50}	Dittmer, 1959
	Mice	467	Oral	LD_{50}	MacEwen and Vernot, 1972

Table 104. Acute Animal Toxicity of Various Nitrophenol Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effect	Reference
p-Nitrophenol (Cont'd)	Mice	107.6	I.P.	LD_{50}	Lawford *et al.*, 1954
	Mice	75	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
	Dogs	10	I.V.	Lethal dose	Dittmer, 1959
Picric acid	Cats	500 mg*	Oral	Nausea, vomiting, fatigue, pain, increased reflex excitability within 45 minutes, tonic and clonic convulsions, ascending paralysis causing death by respiratory failure	VonOettingen, 1941
	Dogs	60	?	Slowing of respiration and heart beat; death by respiratory paralysis	VonOettingen, 1941
	Rabbits	400 mg*	?	Convulsions and death in 3 hours	VonOettingen, 1941
	Rabbits	140-250 mg*		Lowered body temperature, slowing of heart rate, rise and subsequent fall in blood pressure	VonOettingen, 1941
	Rabbits	350-1000 mg*	Oral	Severe damage to the kidneys	VonOettingen, 1941
3-Trifluoromethyl-4-nitrophenol	Rats	40	I.P.	Lethal dose	Christensen and Luginbyhl, 1974
	Mice	25-50	I.P.	Approx. LD_{50}	Doull *et al.*, 1962
2,4,6-Trinitro-m-cresol	Mice	168	I.P.	LD_{50}; hair became erect, marked shivering, occasional spasms followed by great nervous activity (e.g. running around cage), no marked rigor mortis upon death	Harvey, 1953
	Mice	31	I.P.	Lethal dose	Christensen and Luginbyhl, 1974

* Total dose

Table 105. Acute Animal Toxicity of Various Nitrotoluene Derivatives

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2-Amino-4-nitrotoluene	Mice	1800	Oral	LD_{50}; severe degeneration of liver, kidneys, and spleen	Akahori, 1954
2-Chloro-4-nitrotoluene	Rats	3020	Oral	LD_{50}	Christensen and Luginbyhl, 1974
2-Chloro-6-nitrotoluene	Guinea Pigs	?	Dermal	Mild skin irritation as a 20% solution in water or a 50% solution in ether; not a skin sensitizer	Hanavan, 1975
2,3-Dinitrotoluene	Rats	1122	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1072	Oral	LD_{50}	MacEwen and Vernot, 1972
2,4-Dinitrotoluene	Rats	50	Oral	No mortality produced	Hanavan, 1975
	Rats	268	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rats	200 ppm	Inhalation	No mortality produced when inhaled for one hour	Hanavan, 1972
	Mice	1625	Oral	LD_{50}	MacEwen and Vernot, 1972
	Rabbits	200	Topical	No mortality produced; not corrosive to skin	Hanavan, 1975
	Cats	27	Oral	Minimum lethal dose	Spector, 1956
2,5-Dinitrotoluene	Rats	707	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1231	Oral	LD_{50}	MacEwen and Vernot, 1972

Table 105. Acute Animal Toxicity of Various Nitrotoluene Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
2,6-Dinitrotoluene	Rats	177	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1000	Oral	LD_{50}	MacEwen and Vernot, 1972
	Cats	60	I.P.	Lethal dose	Spector, 1956
2,4- & 2,6-Dinitrotoluenes (mixed)	Rabbits	1000	Topical	Approximate lethal dose; not corrosive to skin	Hanavan, 1975
3,4-Dinitrotoluene	Rats	1072	Oral	LD_{50}	MacEwen and Vernot, 1972
	Mice	1414	Oral	LD_{50}	MacEwen and Vernot, 1972
m-Nitrotoluene	Rats	2282	Oral	LD_{50}	Hanavan, 1975
	Rats	1072	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	200 ppm	Inhalation	No mortality produced when inhaled for one hour	Hanavan, 1975
	Mice	330	Oral	LD_{50}	Kosachevskaya, 1967
	Rabbits	2400	Oral	LD_{50}	Kosachevskaya, 1967
	Rabbits	20	Topical	No mortality produced; not corrosive to skin	Hanavan, 1975
	Guinea Pigs	3600	Oral	LD_{50}	Kosachevskaya, 1967

Table 105. Acute Animal Toxicity of Various Nitrotoluene Derivatives (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Effects	Reference
o-Nitrotoluene	Rats	2144	Oral	LD_{50}	Hanavan, 1975
	Rats	891	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	200 ppm	Inhalation	No mortality produced when inhaled for one hour	Hanavan, 1975
	Mice	2462	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rabbits	200	Topical	No mortality produced; not corrosive to skin	Hanavan, 1975
p-Nitrotoluene	Rats	2144	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	2144	Oral	LD_{50}	Hanavan, 1975
	Rats	939.4	I.P.	100% mortality within 24 to 48 hours; 23.6 methemoglobin formation	Magos and Sziza, 1958
	Rats	200 ppm	Inhalation	No mortality produced when inhaled for one hour	Hanavan, 1975
	Mice	1231	Oral	LD_{50}	Christensen and Luginbyhl, 1974
	Rabbits	200	Topical	No mortality produced; not corrosive to skin	Hanavan, 1975
3-Nitro-α,α,α-trifluorotoluene	Rats	610	Oral	LD_{50}	Christensen and Luginbyhl, 1974
Trinitrotoluene	Rats	>700	S.C.	Lethal dose	Spector, 1956
	Rabbits	500-700	S.C.	Lethal dose	Spector, 1956
	Cats	480	Oral	Lethal dose	Spector, 1956
	Cats	200	S.C.	Lethal dose	Spector, 1956

Table 106. Acute Animal Toxicity of Miscellaneous Nitroaromatic Compounds

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
2-*tert*-Butyl-4,6-dinitrophenyl acetate	Rat	62	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
	Rat	>2000	Dermal	LD_{50}	Berg, 1972
2-*sec*-Butyl-4,6-dinitrophenyl-3-methyl-2-butenoate	Rats	58-225	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
	Rats	161 ± 25	Oral	LD_{50}	Berg, 1972
	Rabbits	1350	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
6-*tert*-Butyl-3-methyl-2,4-dinitroanisole (musk ambrette-artificial)	Rats	339	Oral	LD_{50}; increased respiration and hypersensitivity after 24 hours, scrawny fur and appearance, wet posterior; time to death = 1 to 3 days	Jenner *et al.*, 1964
2-*tert*-Butyl-5-methyl-4,6-dinitrophenyl acetate	Rats	42	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
2,5-Dichloro-3-nitrobenzoic acid	Rats	3500	Oral	LD_{50}	Bailey and White, 1965
2,4-Dichlorophenyl-4-nitrophenyl ether (Nitrofen)	Rats	3050	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
2,4-Dinitroanisole	Rats	100	Oral	Lethal dose	National Acad. Sci., 1953
4,6-Dinitro-2-*sec*-butylphenyl acetate	Rats	65	Oral	LD_{50}	Berg, 1972
2,4-Dinitro-6-*tert*-butylphenyl methanesulfonate	Rats	527	Oral	100% mortality	Tsubura and Kato, 1974
	Mice	800	Oral	100% mortality	" "
2,6-Dinitro-N,N-dipropylcumidine (tech)	Mice + Rats	>5000	Oral	LD_{50}	Berg, 1972
	Rabbits + Dogs	>2000	Oral	LD_{50}	" "

Table 106. Acute Animal Toxicity of Miscellaneous Nitroaromatic Compounds (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
2,4-Dinitro-α-naphthol	Rats	47.5	i.p.	LD_{50}	Lawford *et al.*, 1954
	Mice	55	i.p.	LD_{50}	" "
	Dogs	30-60	i.v.	Fatal, death within 30 minutes	Dittmer, 1959
	Guinea pigs	80-100	s.c.	Fatal, death within 15 to 30 minutes	" "
2,4-Dinitro-6-octylphenyl crotonate (Karathane)	Rats	980	Oral	LD_{50}	Berg, 1972
2,4-Dinitrophenetole	Rats	250	Oral	Minimum lethal dose	National Acad. Sci., 1953
2,6-Dinitro-N,N-di-n-propyl-α,α,α-trifluoro-*p*-toluidine (Trifluralin)	Rats	5000	Oral	LD_{50}	Bailey and White, 1965
	Rats	3700-10,000	Oral	LD_{50}	Ben-Dyke *et al.*, 1970
	Rats	>5000	Dermal	LD_{50}	" "
	Rabbits	>200	Dermal	LD_{50}	Edson *et al.*, 1964
Dinitroresorcinol	Dogs	190	s.c.	Fatal within 24 hours; oral administration of 1 to 3 grams had no effect	VonOettingen, 1941
3,5-Dinitrotoluamide	Rats	560	Oral	LD_{50}	Christensen and Luginbyhl, 1974
3,5-Dinitro-*p*-toluidine	Rats	>500	Oral	Minimum lethal dose	National Acad. Sci., 1953
3'-Nitroacetophenone	Rats	3250	Oral	50% mortality after 14 days	Smythe *et al.*, 1954
	Rabbits	3.0 mℓ/kg	Dermal	50% mortality after 14 days	" "
	Mice	200-300	i.p.	Approx. LD_{50}	Doull *et al.*, 1962
5-Nitro-*o*-anisidine	Rats	704	Oral	LD_{50}	Christensen and Luginbyhl, 1974
o-Nitroanisole	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
p-Nitroanisole	Rabbits	?	Dermal	Not corrosive to skin	" "

Table 106. Acute Animal Toxicity of Miscellaneous Nitroaromatic Compounds (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
p-Nitrobenzaldehyde	Rats	545	i.p.	LD_{50}	Christensen and Luginbyhl, 1974
	Rats	619	i.p.	100% mortality; death within 2 to 3 hours; a dose of 420 mg/kg produced a methemoglobin level of 50%	Magos and Sziza, 1958
o-Nitrobenzamide	Mice	500	i.p.	Lethal dose	Christensen and Luginbyhl, 1974
p-Nitrobenzenesulfonamide	Rats	500	Oral	Lethal dose	" "
m-Nitrobenzoic acid	Rats	1820	Oral	LD_{50}; hyperexcitability, immobilization, aggressiveness	Caujolle et al., 1966
	Rats	670	i.p.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Rats	680	i.v.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	1290	Oral	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	610	i.p.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	640	i.v.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
o-Nitrobenzoic acid	Mice	3100	i.p.	LD_{50}; hyperexcitability, immobilization, aggressiveness	Caujolle et al., 1966
	Mice	1920	i.v.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
p-Nitrobenzoic acid	Rats	1960	Oral	LD_{50}; hyperexcitability, immobilization, aggressiveness	Caujolle et al., 1966
	Rats	1210	i.p.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	1470	Oral	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	770	i.v.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
	Mice	880	i.p.	LD_{50}; hyperexcitability, immobilization, aggressiveness	" "
o-Nitrobiphenyl	Rats	1230	Oral	LD_{50}; survival time was 24 hours to 18 days	Deichmann et al., 1947
	Rabbits	1580	Oral	LD_{50}; survival time was 24 hours to 16 days	" "
p-Nitrobiphenyl	Rats	2230	Oral	LD_{50}; survival time was 24 hours to 10 days	Deichmann et al., 1947
	Rabbits	1970	Oral	LD_{50}; survival time was 48 hours to 7 days	" "
1-Nitronaphthalene	Dogs	670	Oral	Approximate lethal dose	Hanavan, 1975
	Rats	120	Oral	LD_{50}	Christensen and Luginbyhl, 1974

Table 106. Acute Animal Toxicity of Miscellaneous Nitroaromatic Compounds (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Adminis.	Effects	Reference
2-Nitronaphthalene	Rats	4400	Oral	LD_{50}; minimum lethal dose was 3200 to 4700 mg/kg; degeneration of liver and kidneys	Treon and Cleveland, 1960
	Rabbits	2650	Oral	LD_{50}; minimum lethal dose was 1400 to 2100 mg/kg; conjunctival discoloration occurred within several hours, urine discolored, severe hepatic and renal degeneration, formation of Heinz bodies and methemoglobinemia	" "
α-Nitrostilbene	Mice	500	i.p.	100% mortality; no lethal effects up to 250 mg/kg	Dittmer, 1959
5-Nitro-o-toluidine	Rabbits	?	Dermal	Not corrosive to skin	Hanavan, 1975
	Rats	574	Oral	LD_{50}	Christensen and Luginbyhl, 1974
3-Nitro-p-toluidine	Starling	32	Oral	LD_{50}	Schafer, 1972
	Blackbird	3.2	Oral	LD_{50}	" "
4-Nitro-2,6-xylenol	Mice	500	i.p.	Lethal dose	Christensen and Luginbyhl, 1974
Tetranitroxylene	Dogs	5000	s.c.	Minimum lethal dose	Christensen and Luginbyhl, 1974
	Rabbits	1000	i.p.	Minimum lethal dose	" "
2,4,6-Trinitroanisole	Rats	>500	Oral	Lethal dose	National Acad. Sci., 1953

processes occurring in the intact cell or animal may have prevented sufficient quantities of DNP from entering the brain mitochondria, thus explaining its lack of uncoupling activity. This argument seems plausible when one considers the studies of Doggett and Spencer (1973), which demonstrated that DNP injected directly into the cerebral ventricles of the brain in mice and rats caused an uncoupling of oxidative phosphorylation. Furthermore, DNP, when administered into the brain, can selectively reduce conditioned avoidance-response in rats with no effect on unconditioned responses. This action of DNP on the brain resembles that of chlorpromazine when centrally administered; this compound is a known uncoupler in brain mitochondria.

In addition, it was found that dinoseb was an inhibitor of mitochondrial respiration but not an uncoupler of oxidative phosphorylation. The symptoms of poisoning by dinoseb differed greatly from those produced by the uncouplers, most notably in that death was not followed by immediate rigor mortis.

2. Subacute and Chronic Toxicity

For the sake of consistency in the following discussion and in Table 107, all toxicity studies which involved more than a single dose and lasted longer than 24 hours, but less than 90 days, will be referred to as subacute. This classification covers the great majority of studies which were obtained in preparing this section. Very few reports were found of studies where nitroaromatic compounds were repeatedly administered for more than three months.

The material presented in this section should be interpreted with special consideration for the fact that target organs and responses of

Table 107. Subacute and Chronic Animal Toxicity

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
2-Cyclohexyl-4,6-dinitrophenol	Rats	0.10%	Dietary	6 months	45% mortality; significantly decreased weight gain	Spencer et al., 1948
	Ducklings	0.25%	Dietary	4 days	100% mortality; a 0.10% diet produced a 90% mortality within 38 days	Spencer et al., 1948
2,6-Dichloro-4-nitroaniline	Dogs	24-48	Dietary	Daily for 2 years	Irreversible cataracts developed after 55 days at the 48 mg/kg level and several weeks later at the 24 mg/kg level; slight elevation of bilirubin and serum glutamic oxaloacetic transaminase levels at the higher dosage level; no other abnormalities were observed; natural light was essential for cataract formation	Bernstein et al., 1970
	Dogs and Swine	192	Dietary	Daily	All dogs died within 49 to 53 days and were extremely emaciated; hemoglobin, hematocrit, and red blood cell count were decreased; Heinz bodies and reticulocytosis were detected in male animals; swine were not affected in any manner	Earl et al., 1971
	Dogs and Swine	48	Dietary	Daily	Irreversible corneal and lens opacities were seen in dogs within 53 to 55 days; Heinz bodies were found in dogs and swine; natural light was essential for eye lesion formation; at a dose level of 24 mg/kg/day some dogs developed eye lesions in 84 to 104 days, while at 75 mg/kg/day changes were observed in 13 days	Earl et al., 1971
	Monkeys	160	Oral	Daily	Lethal within 3 months; more toxic to females than males; caused structural alterations of the liver and kidneys	Serrone et al., 1967
	Rats	400 and 1,000	Oral	Daily for 3 months	Some mortality at only the 1,000 mg/kg level; liver enlargement noted along with increased hepatic demethylase and desulfurase activities; caused structural alterations of liver and kidneys, and increased mitochondrial oxygen utilization	Serrone et al., 1967

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
2,6-Dichloro-4-nitroaniline (continued)	Rats	100 ppm	Dietary	Daily for 3 generations	No adverse effects noted on rat reproduction, number of pups per litter, number of litters per group, stillbirth rates, mean birth and weanling weights, or weanling survival	Johnston et al., 1968
	Dogs	3,000 ppm	Dietary	Daily for 104 weeks	One dog died at 74 weeks; weight gain and clinical chemistry values were normal in all surviving dogs; histologic changes were seen in the liver and yellowing of the skin and mucosa was noted; dose levels below 3,000 ppm produced no mortality	Johnston et al., 1968
	Rats	20, 100, and 3,000 ppm	Dietary	Daily for 104 weeks	Survival was 67-80%, 50-67%, and 43-57% in the high, middle, and low groups respectively; most deaths occurred after 70 weeks; mean body weights of the rats fed 3,000 ppm were 72-75% of controls at 104 weeks; increased organ-to-body weight ratios were seen for the liver, kidneys, testis, and thyroid in the 3,000 ppm group	Johnston et al., 1968
m-Dinitrobenzene	Dogs	0.2-6	S.C.	Long periods	Anemia, Heinz body formation, cramps, paralysis, decreased hemoglobin, increased urobilinogen in the urine, 30% incidence of liver damage	Kiese, 1949
4,6-Dinitro-o-sec-butylphenol	Rats	0.05%	Dietary	21 days	40% mortality; rapid weight loss, slight renal degeneration; rats fed a 0.02% diet for 6 months showed only diminished weight gain	Spencer et al., 1948
	Ducklings	0.10%	Dietary	4 days	100% mortality within 4 days; one animal developed cataracts at day 3; a 0.25% diet produced 100% mortality within 3 days and no cataracts were formed; a 0.03% diet produced 50% mortality within 4 days and cataracts developed in 2 animals at days 5 and 8	Spencer et al., 1948

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
4,6-Dinitro-o-sec-butylphenol (continued)	Mice	16, 40, and 100	Dermal	5 days weekly for 2 weeks	Mice given the 40 and 100 mg/kg doses all died after the first application; those given 16 mg/kg all survived and grew normally	Bough et al., 1965
4,6-Dinitro-2-(2-capryl)phenyl crotonate	Rats	50 and 100	Oral	Daily for 10 days	Lowered protein utilization; decreased feed intake and body weight gain; no effect on net energy utilization	Salmowa et al., 1974
4,6-Dinitro-o-cresol	Rats	0.10%	Dietary	10 days	50-60% mortality; rapid weight loss, enlarged spleen, slight renal degeneration; rats fed a 0.05% diet for 6 months showed only decreased weight gain	Spencer et al., 1948
	Ducklings	0.25%	Dietary	2 days	100% incidence of bilateral cataracts within 24 hours; 100% mortality within 2 days	Spencer et al., 1948
	Rabbits	3% solution in 95% EtOH	Dermal	Daily for 7 days	100% mortality	Spencer et al., 1948
	Rats	20	S.C.	Daily for up to 6 weeks	31% mortality within 17 days when animals were kept in a warm laboratory; 8.5% mortality within 17 days was obtained when rats were kept under cool environmental conditions	Parker et al., 1951
2,4-Dinitrophenol	Rats	0.20%	Dietary	24 days	40% mortality; emaciation, enlarged spleen, slight renal degeneration, liver congestion, testicular atrophy, cloudy swelling of the liver	Spencer et al., 1948
	Rats	0.10%	Dietary	6 months	Decreased rate of body weight gain by 10 to 15%	Spencer et al., 1948

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
2,4-Dinitrophenol (continued)	Rats	20	I.V. and I.P.	Twice daily for 3-4 days	Urine volume increased by up to 3.5 times within 12 hours and lasted 5-7 days; renal damage included dilation of proximal tubules with some dehydrated and necrotic cells; tubular reabsorption was depressed, presumably due to metabolic failure	Eisenbach *et al.*, 1967
	Ducklings	0.25%	Dietary	35 days	100% incidence of bilateral cataracts within 24 hours; 40% mortality after 35 days	Spencer *et al.*, 1948
	Guinea Pigs	10	I.P.	Daily	Animals fed a vitamin C-deficient diet developed cataracts in 14 to 18 days; animals given vitamin C supplementation did not develop cataracts from DNP	Ogino and Yasukura, 1957
3,5-Dinitro-*o*-toluamide	Rats	0.03%	Dietary	90 days	Minimum ill-effect level; the maximum no-effect level was 0.01%	Weil and McCollister, 1963
	Rats	0.0125%	Dietary	2 years	Minimum ill-effect level; the maximum no-effect level was 0.0062%	Weil and McCollister, 1963
2,4- and 2,6-Dinitrotoluenes (mixed)	Rabbits	1,000	Dermal	10 doses over 2 weeks	No cumulative toxicity	Hanavan, 1975
p-Nitroaniline	Rats	5 mg/m^3	Inhalation	5 hours daily for 4 months	Decreased hemoglobin level and erythrocyte count	Vasilenko *et al.*, 1974
p-Nitroanisole	Mice	0.01-0.03 mg/l	Inhalation	2 hours daily, 6 days weekly for 12 months	After 12 months anemia, reticulocytosis, and decreased erythrocyte count were seen; no effects were noted with exposure for one month	Zaeva and Fedorova, 1962

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
Nitrobenzene	Rabbits and Guinea Pigs	0.1-50	Oral	Daily	Doses above 1 mg/kg produced decreases in hemoglobin level, erythrocyte and lymphocyte counts, and increased leucocytes; high doses caused organ degeneration, especially the heart, liver, and kidneys	Kazakova, 1956
	Rats	0.08-0.8 mg/m^3	Inhalation	Continuous for 73 days	Disturbance of muscular chronaxies, increased blood cholinesterase activity, increased methemoglobin levels followed by a persistent increase in sulfhemoglobin; rats exposed to 0.008 mg/m^3 were not affected	Rusakov *et al.*, 1973
	Rats	100-200	S.C.	Daily	Sulfhemoglobinema, anemia, methemoglobinemia, and Heinz body formation	Zvezdai, 1972
	Rabbit	840	S.C.	Daily for 24 weeks	7 of 8 rabbits died within 14 weeks; symptoms observed were emaciation, recurrent anemia, degeneration of the liver, spleen, and adrenal cortex	Yamada, 1958
	Guinea Pigs	200	S.C.	Every other day for 6 months	Fluctuations of urinary 17-oxo-corticosteroids, hemolytic anemia, loss of body weight, decreased motor activity	Makotchenko and Akhmetov, 1972
p-Nitrochlorobenzene	Cats	2.8-25.9 ppm	Inhalation	7 hours a day, 5 days a week	At 25 ppm 1 cat died after 7 hours and 1 survived; at 6.5 ppm 1 cat died after 74 hours; at 3.3 ppm 1 cat died after 24 hours; at 2.8 ppm 1 cat survived after 198 hours of exposure; all animals lost weight; methemoglobinemia was seen and anemia developed in the 2 cats which had long exposures; damage to the liver and kidney was noted in the long exposure animals	Watrous and Schulz, 1950
	Rats	100	S.C.	Daily for 10 days	Sulfhemoglobinemia, anemia, methemoglobinemia, and Heinz body formation	Zvezdai, 1972

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
p-Nitrochlorobenzene (continued)	Rabbits	100	S.C.	Every 4th day for 3-6 months	Resistance to methemoglobinemia and Heinz body formation increased markedly with time	Nogawa, 1961
	Rats	135	Oral	10 doses	Fatal to 2 out of 6 rats	Hanavan, 1975
	Rabbits	0.005, 0.05, and 0.5	?	4 months	Decreased immunological reactivity at the 0.5 mg/kg dosage; definite cumulative properties	Cherkinski et al., 1947
o-Nitrobiphenyl	Rats and Rabbits	0.04 mg/l (dust)	Inhalation	7 hours per day on 62 days over a 165 day period	Irritation of the nasal mucous membrane and decreased body weight gain in rats; no effects on rabbits	Deichmann et al., 1947
	Rats and Rabbits	0.5 mg/l (vapor)	Inhalation	7 hours per day on 17 days	2 of 3 rabbits and 2 of 8 rats died; pulmonary lesions and loss of body weight were also observed	Deichmann et al., 1947
	Rats and Mice	0.008 mg/l (vapor)	Inhalation	7 hours per day on 30 days	Rats developed only mucous membrane irritation; 4 of 8 mice died and pulmonary lesions were noted in this group	Deichmann et al., 1947
	Rats and Mice	0.003 mg/l (vapor)	Inhalation	7 hours per day on 78 days	No mortality; no mucous membrane irritation; mild pulmonary lesions	Deichmann et al., 1947
	Rabbits	500	Dermal	5 days a week for 7 weeks	No effects on skin or body weight	Deichmann et al., 1947

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
p-Nitrobiphenyl	Rabbits	500	Dermal	5 days a week for 7 weeks	No effects on skin or body weight	Deichmann et al., 1947
p-Nitrophenol	Guinea Pigs	2.5	I.P.	Daily	Cataracts developed in vitamin C-deficient animals in 7 to 11 days	Ogino and Yasukura, 1957
Pentachloronitrobenzene	Rats	500 ppm	Dietary	3 generations	No effects seen on reproductive fertility, gestation, viability, or lactation	Borzelleca et al., 1971
	Rats	63.5-5,000 ppm	Dietary	3 months	Animals at the 5,000 ppm level were sacrificed after 2 weeks due to poor health; growth was depressed at levels above 2,500 ppm for females and 1,250 ppm for males; a significant liver-to-body weight ratio increase was seen at all levels except in females at 63.5 ppm; no hematologic changes were seen	Finnegan et al., 1958
	Dogs	5-1,080 ppm	Dietary	2 years	No mortality; lowered hematocrit values after 18 months in dogs on the 30 and 180 ppm diets only; at the 1,080 ppm level higher serum glutamic oxaloacetic transaminase seen in females with elevated SAP levels and slightly enlarged livers in both sexes	Borzelleca et al., 1971
Picric acid	Rabbits	60 mg (Total dose)	Oral	Daily	Jaundice, diarrhea, loss of weight; repeated doses of 180 mg/kg caused severe emaciation and death within 2 weeks	VonOettingen, 1941
	Dogs	1.8	?	Repeated	Kidney damage and increased nitrogen excretion	VonOettingen, 1941

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
Tetryl	Rats and Rabbits	1,000-2,000	Oral	Daily	Caused death within 10 to 18 days; severe degeneration of liver and kidneys	Parmeggiani et al., 1956
	Rats	50	Oral	Daily for 3 months	Slight degenerative changes of the liver and kidneys; a single one gram dose produced no effect	Parmeggiani et al., 1956
Trinitrobenzene	Dogs	100	Oral	Daily for 4 days	Female dog developed convulsions, anorexia, soft feces, alterations of erythrocyte morphology, 11.4% methemoglobin; male dog developed 21.8% methemoglobin; treatment was discontinued due to poor health of dogs	Fogleman et al., 1955
	Dogs	25	Oral	37 doses over 54 days	Female dog developed anorexia, depression, and emesis during the first 30 days but recovered thereafter; male developed generalized muscular stiffness with ataxia after 34 days which persisted to end of the study; maximum methemoglobin levels of 22.4% in the female and 33.8% in the male; spleeen and liver were enlarged and signs of kidney degeneration were present in both male and female	Fogleman et al., 1955
Trinitrotoluene	Dogs	0.1-1.0 and 5-20	S.C.	1.5 years	Disruption of pancreas exacrinous function; changes in pancreatic secretion volume and activities of trypsin, amylase, and lipase	Kleiner, 1971
	Dogs	0.1 or 5-20	S.C.	Every other day for up to 2.5 years	Elevated levels of ammonia, phosphates, and lactic acid in the gastric juice	Kleiner, 1969
	Dogs, Rats, and Rabbits	5-20	S.C.	Every other day for 2 years	Changes in the external secretory activity and incretory function of the pancreas; dystrophic changes in the acinic and incretory apparatus of the pancreas	Kleiner et al., 1974

Table 107. Subacute and Chronic Animal Toxicity (Cont'd)

Compound	Species	Dose (mg/kg)	Route of Administration	Period of Exposure	Effects	Reference
Trinitrotoluene (continued)	Dogs	20-50	S.C.	Every other day for 3 months	Decreased bile secretion and increased cholic acid and bilirubin concentrations in the bile; during the third month cholic acids decreased and cholesterol increased	Kleiner, 1971 b
	Dogs	?	Dermal and Inhalation	Up to 2 years	Disruption of the secretory and evacuating functions of the stomach; changes in the volume of gastric secretion; acid and enzyme-forming functions displayed a wave-like character	Kleiner, 1972
	Rats	100	Oral	3-30 days	Decreased levels of total protein and albumin and increases in β- and γ-globulins in the blood serum; protein-fatty dystrophy of the liver; decreased level of serotonin and increased activity of monoamine oxidase in the liver and brain	Mul'menko and Levina, 1974
	Rats	10	Oral	Daily for 100 days	Decreased leukocyte phagocytic activity which was antagonized by treatment with 50 μg/kg vitamin B_{12} or 5 mg/kg vitamin PP for the first 40 days of TNT administration	Kuzovleva _et_ _al_., 1973
	Rats	100	Oral	Daily for 45 days	Decreased leukocyte phagocytic activity which was antagonized by treatment with 50 μg/kg vitamin B_{12} or 5 mg/kg vitamin PP for the first 40 days of TNT administration	Kuzovleva _et_ _al_., 1973

acute bioassays may not be the same following repeated exposure to smaller doses. The phenomena of storage, metabolic activation, and repeated damage to organs and organelles are potential hazards of repeated exposure; they more closely resemble the consequences to man from environmental contamination.

a. Dinitrophenol Derivatives

The possibility of chronic exposure to low levels of the dinitrophenol compounds is relatively high, based on the patterns of agricultural use for these substances. Recognizing this possible danger, several investigators have undertaken to determine if long-term toxic effects can be produced in laboratory animals.

The results of studies where DNP, DNOC, dinoseb, and cyclohexyldinitrophenol were injected or fed in the diet indicated that cumulative toxic effects did not occur at dosage levels below those which produce toxicity as a single dose (Table 107). Symptoms of severe poisoning could be produced, however, when DNOC was given by repeated injection with short intervals between consecutive doses (Parker et al., 1951). A single dose in the rat or rabbit of 5 mg/kg of body weight produced no deleterious effects, but hourly injections of the same dose caused marked symptoms after the fourth or fifth injection. The further observation was made that among rats given a long series of daily 20 mg/kg injections of DNOC, almost all deaths occurred after the first few injections. This left a group of animals that were either functionally resistant or had developed a tolerance to the effects of DNOC.

It seems evident from the above results and from the data reported in Section III-B that DNP derivatives can be quickly eliminated from the body with little or no cumulative poisoning occurring at dosages below the threshold for acute toxic responses. Furthermore, the apparent susceptibility of some animals and the resistance of others may simply be a reflection of individual variation in the rate of emzymatic detoxification of the administered substance. Many of the reports encountered in preparing this review have demonstrated that considerable inter- and intraspecies variations exist in susceptibility to the toxic actions of DNP and its related compounds.

The potential for cumulative toxicity by the dinitrophenols seems to be significant only for the alkylated derivatives, according to a report by Burkatskaya (1962). He administered DNP, DNOC, dinoseb, and dinitro-*o*-propylphenol to rats and cats by single oral dose of from 10 to 100 mg/kg body weight. He found that DNP was completely eliminated within 24 hours, and that DNOC cleared from the organs in three days and from the blood within five days. Dinoseb was retained for at least five days, and dinitroro-*o*-propylphenol persisted for ten days.

One report has stated (Makhinya, 1969) that, unlike the dinitrophenols, the *ortho*-, *para*-, and *meta*-mononitrophenols have distinct cumulative properties. With these compounds, chronic administration to warm-blooded animals caused alterations of neurohumoral regulation and pathological changes including gastritis, enteritis, colitis, hepatitis, neuritis, and hyperplasia of the spleen. Limiting doses were established for the disruption of conditioned reflex activity and set at 3 mg/kg for *ortho*- and *meta*-nitrophenol, and 1.25 mg/kg for *para*-nitrophenol.

(i) Chronic Cataract Development

An investigation of cataract development in guinea pigs by subacute administration of various nitrophenols was conducted by Ogino and Yasukura (1957). This phenomenon was studied with respect to the effects of a vitamin C-deficient diet upon susceptibility to cataract formation. It was found that the daily oral intake of 10 mg of DNP could produce cataracts in vitamin C-deficient guinea pigs within 14 to 18 days. Control animals, however, which received ascorbic acid supplements along with the DNP-treatment did not develop eye lesions. Among the other compounds tested, it was demonstrated that cataracts could be formed by subacute administration of *p*-nitrophenol, 2-nitro-4-aminophenol, 2-amino-4-nitrophenol, and 4-nitro-6-cyclohexylphenol. Both *ortho*- and *meta*-nitrophenol were inactive as cataractogenic agents.

A cataractogenic agent excreted in the urine of rabbits given DNP orally was identified as 2,4-diaminophenol. Vitamin C-deficient guinea pigs developed cataracts after injection for six days with 2-amino-*p*-quinoneimine, the oxidized form of 2,4-diaminophenol. Previous experiments with cataractogenic substances have resulted in various quinoid substances being identified as the proximate cataract-producing agent in animals given naphthalene, tyrosine, and galactose. Therefore, the authors concluded that DNP is metabolized to an active cataract-forming substance by the following scheme:

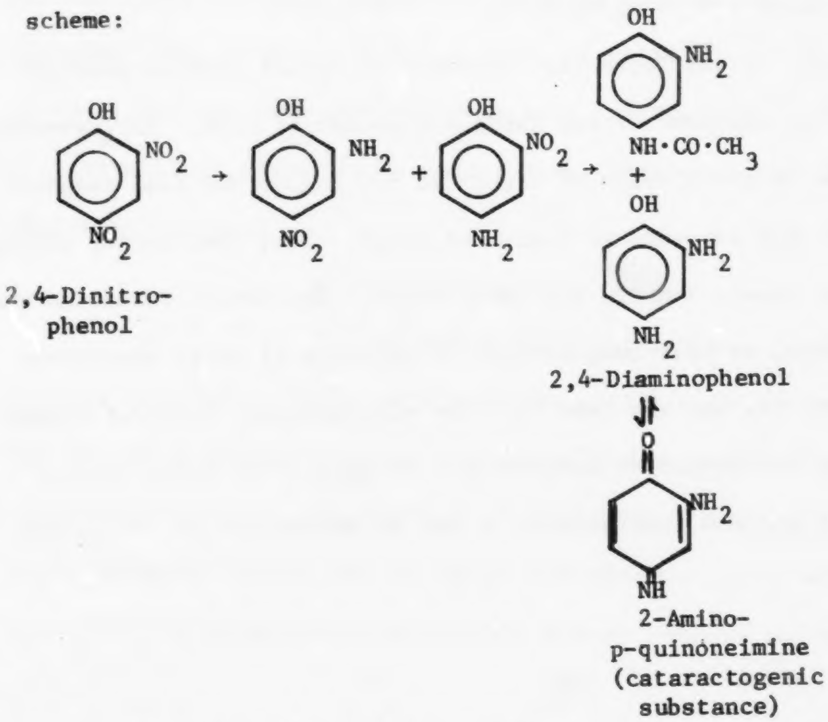


Figure 70. Metabolism of Dinitrophenol and Production of a Cataractogenic Substance (Ogino and Yaskura, 1957)

The exact role of vitamin C deficiency could not be clearly defined in this study. It was shown, however, that the presence of hydroxyl and nitro groups in a para-position seems to be essential for activity.

In addition to the nitrophenols, the compound 2,6-dichloro-4-nitroaniline (DCNA) is also known to produce eye lesions in dogs. Although toxic specificity to the eye is a relatively rare occurrence with most chemicals and drugs, the fungicide DCNA was an effective producer of phototoxic corneal and lens opacities in dogs upon subacute administration (Bernstein et al., 1970). DCNA was fed in the diet of dogs at levels of 0.75, 6.0, 24, 48, or 75 mg per kg of body weight per day. Except for damage to the eye, dose levels up to 48 mg per kg per day produced none of the classical signs of chronic toxicity when dogs were treated for up to two years (Table 107). Irreversible corneal and lens opacities appeared within about 55 days at the 48 mg/kg dosage, and several weeks later at the 24 mg/kg dose level. No abnormalities of any kind were seen at dosages below the 24 mg/kg level.

It was found that exposure to normal outdoor sunlight was essential for the development of eye lesions produced by DCNA. Furthermore, administration of DCNA in the absence of light did not reduce the time required to produce eye damage once exposure to light had begun. This observation indicated that a cumulative drug deposition had not taken place. Eye lesion formation was found to be dose-related, in that long periods of exposure to small quantities of DCNA had no effects. As was the case with the nitrophenols, cataract formation by DCNA seemed to be related to the presence of para-nitro substituent, and possibly an active quinone intermediate formed by metabolism of the parent compound.

Additional investigations by Earl et al. (1971) looked further into the chronic toxicity of DCNA to both dogs and swine. Their findings revealed that DCNA fed at 192 mg/kg/day was lethal to dogs within 49 to 53 days, while dosages of 48 mg/kg/day or below produced no mortality. Swine were not affected in any way, regardless of dose level. Corneal opacities were observed in dogs within 77 days when given DCNA at 48 mg/kg/day and exposed to sunlight. Changes in the eye could be seen in as little as 13 days when animals were given 75 mg/kg/day, thereby indicating a dose-related response. This compound provides a good example of a case where the determination of mortality or LD_{50} values is not sufficient to characterize its true toxic potential.

b. Nitroaniline

In contrast to the apparent non-cumulative properties of DNP derivatives in animals, compounds of the nitrobenzene series can display very pronounced cumulative effects. Vasilenko et al. (1974) noted that mononitroanilines had weak cumulative effects, whereas the introduction of two chlorine atoms into the molecule would increase their cumulative properties. In addition, mononitroanilines were both hemotoxic and hepatotoxic, while dinitroanilines could act as respiratory inhibitors and uncouplers of oxidative phosphorylation.

c. Chloronitrobenzenes

The chloronitrobenzenes are also known to be cumulative poisons (Davydova, 1967), with the most active compound being the para-isomer and the least active compound the ortho-isomer. Aside from their cumulative toxicity to animals, the monochloronitrobenzenes have also been investigated for allergenic action, due to their similarity with 2,4-dinitrochlorobenzene, the extremely potent skin sensitizer in both animals and man (see Section III-B-4).

Rusakov _et al_. (1973) exposed rats to concentrations of _ortho_- and _para_-chloronitrobenzene in air at levels which had been detected in the vicinity around industrial plants. Animals were made to inhale, over a five month period, either the _para_- or _ortho_- isomer at a concentration of 0.008 mg/m^3. A third group was subjected to a combination of both substances, each present at 0.008 mg/m^3. The state of sensitization was determined by the presence or absence of circulating antibodies at the end of the treatment. The results demonstrated that _para_-chloronitrobenzene produced a strong state of sensitization, as also did the mixture of both isomers. _ortho_-Chloronitrobenzene produced sensitization as well but was the least potent substance in that regard. The authors found that it was possible to achieve a passive transfer of allergy by injecting a leucocyte mass and blood serum from sensitized rats into the skin of recipient guinea pigs.

Both the _ortho_- and _para_-isomers of chloronitrobenzene were also established as contact skin sensitizers, although their activity was considerably less than 2,4-dinitrochlorobenzene. It was noted that three drops of a one percent solution of 2,4-dinitrochlorobenzene applied to the skin of guinea pigs would sensitize all guinea pigs tested, whereas the same dose of _ortho_- or _para_-chloronitrobenzene was not effective. Increasing the concentration to 10 percent resulted in sensitization of all animals receiving _para_-chloronitrobenzene but was effective in only half of those treated with _ortho_-chloronitrobenzene.

d. Nitrobenzene

A comprehensive study on the effects of chronic poisoning by nitrobenzene in rabbits was undertaken by Yamada (1958). His observations amply illustrate the pronounced development of toxic symptoms that occurs with increasing time of nitrobenzene exposure.

Daily subcutaneous injections of 0.7 ml (840 mg) per kg body weight of nitrobenzene were given to eight rabbits over a six month period. This treatment evoked a three stage response; an initial response stage, a resistance stage, and a final exhaustion stage. Anemia occurred during the first stage which disappeared during the resistance stage, only to reappear again before death. Increasing reticulocyte counts progressed throughout the entire experiment. In the last stage of poisoning, a breakdown of metabolic detoxification processes was evidenced by reduced capability for amination, acetylation, and hydroxylation reactions, and heavy output of urinary metabolites. Loss of appetite and emaciation occurred during the final stage of poisoning, and extensive histopathological damage was noted (Table 108).

As was the case with chronic poisoning by the dinitrophenols, several of the nitrobenzene-treated rabbits died early in the experiment, while most of the remaining animals survived until being sacrificed after six months. Pathological comparisons of the animals dying early with those that survived revealed that the only difference was in damage to the adrenal cortex. It is not clear whether individual differences in adrenal function can account for variation in resistance to chronic nitrobenzene poisoning. However, the role of the adrenal gland in response to environmental stresses and adaptation through compensatory physiological mechanisms is clearly vital to survival.

Table 108. Chronic Effects of Subcutaneous Nitrobenzene Injections on Tissues of the Rabbit (Yamada, 1958)

Liver	Yellowish appearance, intense stasis. Fatty degeneration, irregular cellular cord, turbid swelling and karyolysis of liver cells. Slight hyperplasia of connective tissues, considerably severe cellular infiltration. These findings are slight in degree in 3 of 8 rabbits.
Kidney	Swelling and adhesion of glomeruli, scanty fluid in glomeruli, slight cellular infiltration. Slight hyaline droplet degeneration of tubular cell. These findings are more remarkable in 4 of the 8 rabbits.
Spleen	Enlargement is seen only in 3 of the 8 rabbits and which also survived more than six weeks. Intense stasis, opening of sinus and appearance of megakaryocytes, hypertrophy and hyperplasia of reticuloendothelial cells, yellowish brown pigments are seen in many cells. Hypertrophia and hyaline degeneration of vascular wall. Malpighian follicle is clearly observed.
Adrenal	In 5 rabbits: The fascicular layer is narrow in width. Degenerated darkish and small cellular groups with pyknotic nucleus, disorder of cellular cord and the lack of intracellular minute vacuole are seen in the fascicular layer. In 3 rabbits: The findings described above are rare. Formation of submembranous cortical nodules is seen. In both groups of rabbits: Few findings in the reticular and glomerular layer and in the medulla.
Heart	Slight wax and vacuole degeneration, atrophy of muscle.
Lung	Hypertrophy of alveolar wall and megakaryocytes in alveolar vessel are seen only in one rabbit.
Bone-Marrow	Increase in megakaryocytes.
Pancreas	Degeneration of Langerhans' Insule is seen in high grade only in one rabbit.

e. Nitrotoluenes

In terms of chronic toxicity, the mono-, di-, and trinitrotoluenes are probably less hazardous than their corresponding nitrobenzene derivatives. Kovalenko (1973) noted that the hemotoxicity resulting from oral administration of these compounds to rats for one to three months decreased in the order trinitrotoluene > dinitrotoluene > m-nitrotoluene > p-nitrotoluene > o-nitrotoluene.

Studies by Shils and Goldwater (1953) indicated that diet was an important factor in the susceptibility to dinitrotoluene poisoning. They found that a high-fat diet increased the resistance of rats to the lethal effects of 2,4-dinitrotoluene when administered by injection but not when it was given in the diet. This discrepancy may have been due to a higher food intake on the high-fat diet, which caused a greater ingestion of the chemical when incorporated in the food. A high-protein diet, on the other hand, reduced the incidence of mortality by 2,4-dinitrotoluene, regardless of the mode of administration.

A previous report by Shils and Goldwater (1950) stated that susceptibility of rats to trinitrotoluene poisoning was not influenced by either the amount or type of fat in the diet. Protein content in the diet was likewise ineffective in altering TNT toxicity.

f. Trinitrotoluene (TNT)

Much of the published work concerning the subacute toxicity of TNT to animals was reviewed by Von Oettingen (1941, 1944), Dacre and Rosenblatt (1974), and Jaffe *et al*. (1973). Early studies indicated that a marked variation in susceptibility to TNT poisoning occurred among the different mammalian species. When administered to dogs, the most significant features of subacute poisoning were anemia and red blood cell destruction, as well as reduced hemoglobin levels and a compensatory increase in the reticulocyte count. The feeding of TNT to dogs also produced ataxia, incoordination, diarrhea, and darkened urine after two to three doses at 5 to 100 mg/kg body weight. Damage to the central nervous system developed with subacute feeding of 50 mg/kg/day for 12 weeks. Repeated injections of TNT to dogs has produced serious disturbances of the gastric and pancreatic secretions, as well as changes in bile secretion (Kleiner, 1969, 1971, 1972; Kleiner _et al_., 1974).

The rat and rabbit are clearly less affected by TNT exposure, although the reason for this is unknown. Rabbits given 200 mg/kg body weight of TNT by subcutaneous injection every other day would survive for 17 to 57 days (Jaffe _et al_., 1973). However, when cats were given daily doses of 50 mg/kg body weight by subcutaneous injection, they died in four to nine days. The treatment of rats with daily oral doses of TNT at 30 mg/kg body weight for six days produced only a decrease in phagocytosis, which could be prevented by niacin administration.

A great deal of further investigation remains to be completed with respect to the toxicity of the munitions-related nitrotoluene derivatives. An extensive series of investigations have been undertaken by the U.S. Army Medical Research and Development Command to determine the toxicity of TNT in different animals by various routes of exposure (Glennon, 1975). These studies are aimed at establishing environmental quality standards for unique munitions water and air pollutants. They include aquatic and mammalian toxicity determinations for TNT and dinitrotoluene, as well as inhalation toxicity studies on the mononitrotoluenes.

3. Sensitization

There is little doubt that several nitroaromatic chemicals are active skin-sensitizing agents. Most notable among this group is 2,4-dinitrochlorobenzene (DNCB), which is considered to be one of the most potent contact allergens and primary skin irritants known to man (see Sections III-B-4-b and III-C-1-e). Documented evidence of accidental human exposures to DNCB is very limited, but controlled studies with humans have demonstrated without question that DNCB is a nearly universal skin sensitizer. Contact with DNCB, even in minute concentrations, must certainly be avoided at all costs.

A recent study (Krawiec and Gaafar, 1975) has compared the allergenic and primary skin-irritant properties of DNCB in dogs. Although dogs are generally resistant to experimental allergic contact dermatitis, sensitization was established in all of 14 pups following DNCB challenge. As expected, seven non-sensitized control pups could not be made to react to a DNCB challenge dose. The pups were sensitized, in most cases, by intradermal injection with 0.1 ml of 0.1% DNCB every other day for a total of 10 injections. All pups were challenged two weeks after the last sensitizing injection by placing six to eight patches containing DNCB on various skin locations. The reactions to DNCB challenge involved slight erythema and edema in two days, moderate erythema and edema after eight days, with severe erythema and edema at the patch application site in two animals.

To determine the primary skin irritation caused by DNCB, closed patches were applied to the ventral skin of non-sensitized dogs, which contained solutions of 1%, 5%, and 10% DNCB in ethanol. Reaction to DNCB was most severe at the 10% dosage and involved complete necrosis and loss of structure of the epidermis after one to five days of exposure. The dermis became edematous, and massive infiltration with polymorphonuclear cells also took place. Microscopically, the lesions of DNCB-induced primary irritant dermatitis were said to be similar to those produced in guinea pigs and man. In contrast to the cellular changes seen in primary skin irritation by DNCB, the lesions of allergic contact dermatitis produced infiltration by mononuclear cells and a much milder dermal edema. The allergic reaction to DNCB reached a peak in intensity three to four days post challenge, and by seven days the inflammatory response had subsided. In primary skin irritation by DNCB, regeneration of the epidermis seemed to begin within 72 hours of the application.

Considerable evidence has been accumulated from occupational studies among munitions factory workers that dermatitis and skin sensitization are common health hazards. Many reports have been made which link exposure to trinitrotoluene (TNT), picric acid, and trinitroanisole to the development of severe dermatitis and skin irritation (Von Oettingen, 1941; Schwartz, 1944). It is difficult to distinguish from the evidence presented, however, whether all cases have resulted from true allergic sensitization or may have involved primary skin irritation as well. Schwartz has stated that sensitization to TNT, ammonium picrate, and picric acid is known to occur in chronically exposed workers. These reactions are most common for TNT at the hands, wrists, and forearms, as well as at points of friction on the body such as the collar and belt lines. With ammonium picrate and picric acid, the site of dermatitis usually involves the face, especially around the mouth and sides of the nose. Edema, papules, and vesicles develop, which are subsequently followed by desquamation.

Allergic sensitization to tetryl is a well-known fact, supported by a number of extensive occupational studies. A study on a working population of 800 to 900 employees engaged in tetryl manufacture (Probst et al., 1944) revealed that four percent of the workers had dermatitis. A review of 404 cases of tetryl dermatitis indicated that age, sex, and race did not affect susceptibility. They observed that most cases occurred among new workers one to two weeks after their introduction to tetryl. This evidence is clearly suggestive of the mechanism of delayed contact hypersensitivity to DNCB, which produces a state of sensitization in humans within two weeks after their initial exposure (see Section III-B-4-b). Schwartz (1944) also reported that tetryl dermatitis was probably the most common cutaneous hazard associated with munitions manufacture. He cited a working population of 6,394 persons exposed to tetryl in which 1,904 (30%) developed tetryl dermatitis in the first six months of operation. Dermatitis cases generally reached a maximum number after about the third week. Most workers could become "hardened" to tetryl one to four weeks after the development of dermatitis and no longer be affected by exposure. In one shell-loading plant, 85% of the workers who had been affected became non-reactive, which may suggest a possible state of chronic immunosuppression, due to continuing tetryl exposure.

An extensive review on tetryl toxicity has been prepared by Bergman (1952) which presented evidence for tetryl dermatitis being due to allergic sensitization rather than local skin irritation. The author summarized the results of animal studies whereby guinea pigs were sensitized to tetryl following intradermal injection, subcutaneous implantation, or smoke inhalation. Animals become reactive to tetryl ten to fourteen days after the initial exposure. It was noted that guinea pigs sensitized to tetryl were also cross-sensitized to picryl chloride and 2,4,6-trinitrophenetole. Several occupational studies reported by Bergman were concerned with the incidence of dermatitis among tetryl workers. Table 109 summarizes ten years of experience at the Picatinny Arsenal and shows that a combined average of 6.05% of exposed workers developed dermatitis.

Table 109. Tetryl Exposure at Picatinny Arsenal; Incidence of Dermatitis Treated (Bergman, 1952)

Year	Exposed	Dermatitis
1941	2,710	5.0%
1942	4,410	7.6%
1943	4,140	7.4%
1944	2,500	7.7%
1945	2,669	6.9%
1946	917	4.8%
1947	507	6.0%
1948	649	5.1%
1949	767	5.8%
1950	1,182	4.2%

Additional studies were cited by Bergman, which revealed the remarkably high prevalence of dermatitis as the principal toxic reaction to tetryl. One report involved 1,258 cases of tetryl poisoning, 75% of which were due to dermatitis alone. Another report was made of 3,807 cases of dermatitis, none of which involved any systemic poisoning.

A few isolated reports of human exposures to various nitroaromatic substances causing allergic dermatitis have appeared in the literature. One incident was noted where a man developed contact dermatitis from handling 3,5-dinitrotoluamide (Bleumink and Nater, 1973; see Section III-C-1). Other reports have described allergic skin reactions in humans to pentachloronitrobenzene (Finnegan et al., 1958; see Section III-C-1-e) and nigrosine (Calnan and Connor, 1972; see Section III-C-1-e). Sensitization may also be achieved by exposure to nitrobenzene and nitrotoluenes (Mayer, 1954). Studies with animals have clearly demonstrated that contact sensitivity may be produced in response to dinitrofluorobenzene (Schneider, 1974), as well as to picric acid and picryl chloride (Chase and Maguire, 1974).

4. Mutagenicity

Information is very limited with regard to the mutagenic potential of most nitroaromatic compounds. Mutagenicity data are particularly important, however, not only in assessing environmental chemicals as potential hazards to reproduction, but also in predicting carcinogenicity. Kriek (1974) has stated that all carcinogens are also mutagenic (but not necessarily vice-versa). Furthermore, the International Agency for Research on Cancer now includes mutagenicity data in its monographs on the evaluation of the carcinogenic risk of chemicals to man.

A study has recently been conducted on the effect of various phenolic compounds, including DNP, on chromosomes of bone marrow cells from mice (Micra and Manna, 1971). Mice were injected intraperitoneally with varying doses of DNP and bone marrow tissue collected 24 hours after treatment. The results, summarized in Table 110, showed that DNP produced mainly chromatid type breaks. There was no linear relationship, however, between the frequency of chromosome aberrations and the dose of DNP.

Table 110. Frequency of Chromosomal Aberrations Induced by Saturated Solution of DNP After 24 Hours of Treatment (Micra and Manna, 1971)

Dose in ml	No. of Metaphases Counted	No. of Metaphases with Aberrations	No. of Chromatid Breaks	% of Aberrations	In Affected Cell Break per Chromosome
0.25	290	20	20	6.9	0.025
0.50	230	34	45	14.5	0.033
1.0	250	42	57	17.2	0.034

The commonly employed herbicide dinoseb acetate was tested for mutagenicity by measuring its effect on the induction of mitotic gene conversions in a diploid strain of the yeast Saccharomyces cerevisiae (Siebert and Semperle, 1974). This test is regarded as a sensitive indicator of compounds which produce base-pair substitutions and frame-shift mutations. The results demonstrated that dinoseb acetate did not significantly increase conversion frequency over the control level.

Using a bacterial mutational system based on reversion to tryptophan independence (try$^+$) in Escherichia coli, Clarke (1971) tested the mutagenic activity of pentachloronitrobenzene (PCNB). He found that PCNB caused a ten-fold increase in try$^-$ to try$^+$ revertant numbers, but only in the hcr$^-$ (excision repair deficient) strain. PCNB was not found to be mutagenic in the hcr$^+$ (excision repair competent) strain.

When tested for mutagenic activity in mice, PCNB did not significantly increase the mutation rate in studies by Busselmaier and coworkers (1973). In addition, they demonstrated that p-nitrophenol was also ineffective as a mutagenic agent in the same test system.

A report abstracted from the foreign literature (Romanova and Rapoport, 1971) detailed the results of treating spores of Actinomyces sphaeroides for two hours with various nitro compounds. Among these compounds were meta- and para-nitroaniline, meta- and para-nitrophenol, meta- and para-nitrobenzaldehyde, ortho-chloronitrobenzene, para-nitrotoluene, and nitrobenzene. At concentrations of 0.001 to 0.004 M these chemicals caused a decrease in viability of 6 to 80%. The greatest number of morphological alterations were caused by meta-nitrobenzaldehyde (25%), and the fewest by para-nitroaniline (12.6%). The nitroaniline isomers and meta-nitrobenzaldehyde produced marked mutagenic effects at 0.001 M.

More recently, mutagenicity tests have been conducted on hair colorants and constituents containing nitrophenylenediamines (Searle et al., 1975). The two compounds, 2-nitro-p-phenylenediamine (2-NPPD) and 4-nitro-o-phenylenediamine (4-NOPD) were tested in bacteria which detected either base-substitution or frame-shift mutations. Negative results were obtained using those bacteria which reverted by base substitution, but both 2-NPPD and 4-NOPD were found to be mutagenic when tested against the frame-shift mutant detecting strains. 4-NOPD was about three times more potent as a mutagen than 2-NPPD at equivalent doses (Table 111).

Table 111. Mutagenicity of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine in *S. typhimurium* TA1538 With or Without Liver Microsomal Activation (Searle *et al*., 1975)

Dose Applied	Induced his^+ Revertants per Plate 5 μg per Plate -(S-9)	 5 μg per Plate +(S-9)	 50 μg per Plate -(S-9)	 50 μg per Plate +(S-9)
Test Compound				
2-NPPD	43	27	335	213
4-NOPD	133	188	883	727

Samples were assayed in the presence or absence of benzo(α)pyrene-induced rat liver supernatant (S-9 mix).

Because many mutagens are known to be chromosome-breaking agents, both 4-NOPD and 2-NPPD were added to cultures of human peripheral blood lymphocytes to test for the production of chromosome damage. In this system, 4-NOPD failed to show any chromosome damage in cultures at concentrations up to 100 μg/ml during incubation for 48 and 72 hours. Similar experiments with 2-NPPD, however, produced a considerable number of chromosome and chromatid gaps and breaks at concentrations between 50 μg/ml and 100 μg/ml (Table 112).

Table 112. Effect of 2-Nitro-*p*-Phenylenediamine on Cultured Human Lymphocytes (Searle *et al.*, 1975)

	Total Cells Examined	Number of Cells with Aberrations		
		B Cells	C Cells	Total Abnormal
2-NPPD present from Time Zero (µg ml^{-1})				
75	100	10	1	11
50	100	8	1	9
25	100	5	0	5
2-NPPD Present for 24 h before Harvesting (µg ml^{-1})				
75	100	21	2	23
50	100	17	0	17
25	100	2	1	3
Control	100	3	2	5

Human peripheral blood lymphocyte cultures were collected at 48 h. Cells were stained with orcein and scored for aberrations but not fully analyzed. B cells have chromosome or chromatid gaps or breaks only, whereas C cells have stable or unstable chromosome rearrangements

5. Teratogenicity

Only a few reports have appeared in the literature concerning teratogenic effects from exposure to nitroaromatic compounds. The concept of teratogenesis used in searching the literature was applied in its broadest sense and included (1) structural and/or functional abnormalities occurring during gestation, (2) embryotoxicity or fetal death and resorption, and (3) fetal growth retardation. Nevertheless, the material which follows in this section is, for the most part, limited in its scope and suggestive of the need for further research.

a. 2,4-Dinitrophenol

Hagstrom and Lonning (1966) conducted a detailed analysis of the morphogenetic effect on the sea urchin embryo of 2,4-dinitrophenol (DNP). In their study, direct observations were made on the relative rates of cleavage and course of development both during and after periods of treatment with DNP.

They found that pretreatment of unfertilized eggs with DNP had little effect on the subsequent development of the egg following insemination. On the other hand, there were several distinct effects on the cells of developing larvae subjected to DNP treatment. At concentrations above 10^{-4}M a cytostatic effect on cleavage takes place, such that cells remain in the interphase portion of mitosis until DNP is removed. Even though mitosis could be reinitiated, developmental irregularities were noted in the embryo, which were dependent upon the duration of DNP treatment. In addition, it was found that the effects of DNP were more persistent on embyros which had been exposed during an early developmental stage (e.g. 16-cell stage) rather than during the late blastula or gastrula phase.

While concentrations of DNP at about 10^{-4}M stopped cell division, development of the larvae progressed even in the continued presence of DNP at concentrations below 10^{-4}M. These larvae, however, showed signs of inhibited differentiation, and development of the gut, skeleton, and arms was inhibited in relation to controls.

Cytological examination of the mitochondria of sea urchin blastomers revealed a definite swelling and aggregation following treatment in DNP. The mitochondria appeared to be paralyzed and lacked the characteristic "jerking" movements in the cytoplasm displayed by controls. Upon removal of DNP, new mitochondria began to form, but the aggregated clusters from the DNP treatment persisted. Pathological changes noted in the development of the larvae exposed to low concentrations of DNP were attributed to the failure of mitochondrial populations to recover from the cytostatic effects of DNP.

A report of teratogenic synergism following the combined administration of DNP and insulin to chicks was made by Landauer and Clark (1964). Insulin is a well-known teratogen, causing various structural abnormalities when administered to chicks during the first two days of incubation. The injection of 100 μg/egg of DNP was non-toxic and non-teratogenic after 96 hours of incubation. However, the combined administration of 1.5 I.U. of insulin with 100 μg of DNP dramatically raised the incidence of embryo mortality and shortened upper beak (Table 113).

Table 113. Results of Experiments in Which Either Insulin or 2,4-Dinitrophenol or Both Compounds Were Injected into the Yolk Sac of Eggs of White Leghorn Fowl After 96 Hours of Incubation (Landau and Clark, 1964)

Insulin (I.U.)	1.5	1.5	--
2,4-Dinitrophenol (μg)	--	100	100
Treated	184	281	185
Mortality percent			
to end sixth day	17,9	53.0	3.2
7-13	3.8	0.7	2.2
14-22	26.1	23.5	18.3
Hatched percent	52.2	22.8	76.2
Survivors of thirteenth day	144	130	175
Normal percent	84.0	70.8	97.7
Micromedia percent	15.3	15.4	0.0
Parrot or short lower beak percent	4.2	3.8	0.0
Short upper beak percent	1.4	18.5	0.0
Miscellaneous defects percent	2.1	3.8	1.7

The combination of insulin with sodium salicylate, another uncoupler of oxidative phosphorylation, also caused great synergistic increases in embryo mortality and other malformations. These results suggest a possible enhancement of teratogenic potency by certain compounds via the concomitant uncoupling of oxidative phosphorylation. It may likewise be postulated that other nitroaromatic uncouplers (e.g., DNOC, nitrosalicylanilides) would also magnify the teratogenic effects of certain substances.

When Gibson (1973) treated pregnant mice during early organogenesis with oral or intraperitoneal doses of DNP, no significant morphologic defects were noted in the fetuses (Table 114).

b. 2-*sec*-Butyl-4,6-dinitrophenol

The teratogenic potential of 2-*sec*-butyl-4,6-dinitrophenol (dinoseb) was studied in considerable detail by Gibson (1973). Dinoseb was administered daily to groups of pregnant mice by intraperitoneal or subcutaneous injection and by oral intubation. Treatments were given either throughout organogenesis (days 8-15 of gestation), during early organogenesis (days 10-12), or during late organogenesis (days 14-16).

When given intraperitoneally at 17.7-20.0 mg/kg/day, dinoseb produced hyperthermia in the dams and some maternal deaths. Those that survived bore litters of smaller number and size than control dams. The incidence of gross soft tissue and skeletal anomalies produced by dinoseb during early organogenesis is given in Table 115. When administered throughout organogenesis at 5 mg/kg/day, dinoseb produced no fetal anomalies; this dose was considered to be the no-effect level.

The subcutaneous administration of dinoseb produced maternal toxicity at similar doses to intraperitoneal injection but dinoseb was not as effective by this route in producing fetal malformations. The no-effect level for dinoseb by this route of administration was found be about 10 mg/kg/day.

Table 114. Effect on Resorption Rate and Fetal Size of 2,4-Dinitrophenol (DNP) Administered to Mice During Early Organogenesis (Days 10-12 of Gestation) (Gibson, 1973)

Dose Level of DNP (mg/kg/day) and Route	Molar Equivalents of Dinoseb (mg/kg)	Number of Pregnant Mice Treated	Number of Implantations	Number of Fetuses†	Resorptions† (%)	Fetal Body Weight† (g)	Fetal Crown-Rump Length† (cm)
0	---	9	13 ± 1	12 ± 1	4.4 ± 2.0	1.409 ± 0.036	2.7 ± 0
7.7 (ip)	10	8	13 ± 1	12 ± 0	5.6 ± 1.8	1.383 ± 0.034	2.6 ± 0
13.6 (ip)	17.7	8	13 ± 1	11 ± 1	14.1 ± 7.1	1.307 ± 0.038*	2.6 ± 0*
25.5 (oral)	32	7	14 ± 1	13 ± 1	9.6 ± 3.0	1.351 ± 0.029	2.7 ± 0
38.3 (oral)	50	7	13 ± 1	12 ± 1	6.1 ± 2.9	1.366 ± 0.037	2.6 ± 0

† Mean response/litter ± SEM.
* Values marked with an asterisk differ significantly from those of controls: *P<0.05.

Table 115. Gross, Soft-Tissue, and Skeletal Anomalies in Offspring of Pregnant Mice Given Dinoseb by I.P. Injection During Early Organogenesis (Days 10-12) (Gibson, 1973)

	Incidence of Anomalies Following Treatment at Dose Levels (mg/kg/day) of					
	0	10.0	12.5	15.8	17.7	18.8
Anomalies No. of litters examined ...	8	11	7	7	13†	6
Gross						
Oligodactyly	0	0	1.3 ± 1.3	0	35.9 ± 9.8*	15.0 ± 13.1
Imperforate anus	0	0	0	2.9 ± 2.9	19.2 ± 8.7*	6.7 ± 6.7
Acaudia	0	0	0	2.9 ± 2.9	17.5 ± 7.7*	1.7 ± 1.7
Microcaudia	0	0	0	0	25.3 ± 8.0*	7.8 ± 6.5
Brachygnathia	0	0	0	0	2.5 ± 2.5	0
Amelia	0	0	0	0	16.3 ± 8.7*	0
Micromelia	0	0	0	0	5.5 ± 3.9	8.3 ± 8.3
Open eyes	0	3.6 ± 3.0	1.1 ± 1.1	8.3 ± 5.9	0	0
Soft-Tissue						
Internal hydrocephalus	14.6 ± 8.4	92.0 ± 3.0*	97.1 ± 2.9*	55.9 ± 11.5*	76.2 ± 8.6*	20.5 ± 5.5
Hydronephrosis	5.4 ± 5.4	15.6 ± 4.5	23.4 ± 6.3	18.9 ± 10.2	31.6 ± 8.1*	18.5 ± 5.7
Cleft palate	1.8 ± 1.8	1.3 ± 1.3	0	0	0	0
Enlarged bladder	3.6 ± 2.4	0	0	9.4 ± 4.6	1.4 ± 1.4	8.2 ± 5.3
Adrenal agenesis	0	0	0	0	16.2 ± 9.9	10.0 ± 10.0
Skeletal						
Ribs: supernumerary	27.2 ± 12.2	13.1 ± 6.8	23.4 ± 13.6	20.7 ± 6.1	26.0 ± 8.9	24.7 ± 5.9
fused	0	0	22.1 ± 9.4	14.3 ± 14.3	54.5 ± 11.6*	37.2 ± 13.9*
absent	0	0	0	0	12.5 ± 7.7*	0
Sternebrae: fused	0	0	0	0	15.6 ± 7.8*	0
absent or not ossified	6.6 ± 4.3	11.2 ± 3.6	19.7 ± 7.2	34.0 ± 13.9	56.5 ± 9.8*	25.8 ± 13.6
Vertebrate: fused	0	0	4.4 ± 2.9	2.0 ± 2.0	76.2 ± 8.1*	37.2 ± 13.9*
not ossified	0	0	0	0	19.5 ± 8.8*	0
absent	0	0	0	7.1 ± 7.1	30.8 ± 10.5*	0
Long bones absent or not ossified	0	0	4.8 ± 3.1	9.1 ± 7.1	41.3 ± 10.7*	13.3 ± 9.9

† Only 12 litters examined for soft-tissue anomalies.

* Values are the mean percentage responses/litter ± SEM and those marked with asterisks differ significantly from those of controls: *P<0.05.

Doses of dinoseb as high as 50 mg/kg/day were given by oral intubation during early organogenesis but had no effect on fetal size or survival. Oral treatment at any stage of gestation did not produce statistically significant gross or soft tissue anomalies, but skeletal defects were noted in some groups at very high doses levels (20-32 mg/kg/day). The no-effect level for teratogenicity and embryotoxicity throughout organogenesis was set at 20 mg/kg/day.

The author concluded that these results indicated a dose-response relationship for teratogenesis by dinoseb which was dependent on the route of administration. Furthermore, a threshold level seemed to exist such that high doses produced terata and a lower dose could be established below which teratogenicity did not occur.

In a later study, Preache and Gibson (1974 a) investigated the effect of maternal food deprivation on teratogenicity by dinoseb. Pregnant mice were deprived of food for 24 hours on the ninth day of gestation and given intraperitoneal injections of dinoseb at 15.8 mg/kg/day for the next three days. Litters from the food-deprived, dinoseb-treated mothers had a higher incidence of anomalies than those from mothers given dinoseb alone. Although the incidence of anomalies was increased, the types of defects produced were qualitatively the same from food-deprived and non-food-deprived mothers.

The effects of environmental stress were also investigated in combination with dinoseb treatment to produce teratogenicity in mice (Preache and Gibson, 1974 b). Dinoseb was administered at 0-17.7 mg/kg/day to pregnant mice on days 10, 11, and 12 of gestation. By forcing the mice to swim for two hours after the day 11 treatment, the incidence of external, soft tissue, and skeletal anomalies was reduced significantly. Reducing the ambient temperature to 4°C for two hour periods had no effect, but raising the temperature to 32°C on day 11 lowered the dose required to produce maternal death and embryo toxicity.

c. Pentachloronitrobenzene

Jordan and Borzelleca (1973) treated pregnant rats by oral intubation of from 100 to 1,562 mg/kg/day of pentachloronitrobenzene (PCNB) on days 6 through 15 of gestation. On day 20, when dams were sacrificed and fetuses removed, examination revealed no significant incidence of skeletal or soft tissue anomalies in PCNB-treated rats as compared to controls.

In another study, however, (Courtney, 1973) PNCB was found to inhibit kidney formation in the mouse fetus. Renal agenesis was produced in 80% of mouse litters by oral administration of 500 mg/kg daily from day 7 to 11 of gestation.

More recently, a reevaluation of PNCB teratogenicity was conducted in the rat by Jordan _et_ _al_. (1975). Pregnant rats were treated during the most teratogen-sensitive period of gestation (days 6-15) with daily oral doses of PCNB at 8, 20, 50, or 125 mg/kg body weight. The treatment had no significant effect on the number or position of implantations, incidence of dead and resorbed fetuses, viable litter size, fetal sex ratios, and birth weights. Fetal examinations were performed on day 20 of gestation, both visually and by histopathologic analysis. External, skeletal, and soft tissue malformations in the offspring of PCNB-treated rats did not differ significantly from the incidence of defects in negative controls.

6. Carcinogenicity

The production of cancer by most nitroaromatic compounds has not been definitely established as an occupational hazard to man. However, a number of nitroaromatic chemicals are active tumor initiators, tumor promoters, or complete carcinogens in various animals. Furthermore, because metabolic conversion of nitro groups to amino, nitroso, and hydroxylamino substituents can occur, nitro compounds which are initially inactive may generate highly carcinogenic substances.

The nitroaromatic compounds which are known to produce tumors fall into three general categories: 1) derivatives of 4-nitroquinoline-N-oxide; 2) certain mono-nitro and heterocyclic nitro compounds such as polychlorinated nitrobenzenes, and derivatives of 5-nitrofuran, 5-nitroimidazole and 4-nitrobenzene; and 3) nitro analogs of classical aromatic amine carcinogens such as 4-nitrobiphenyl, 2-nitrofluorene, 2,7-dinitrofluorene, 4-nitrostilbene, and derivatives of nitronaphthalene.

All of the known tumor-producing nitroaromatic substances appear to owe their activity to a highly reactive metabolic intermediate rather than to the parent compound. Recent studies by Poirier and Weisburger (1974) and Sternson (1975; see Section III-B-3) support the belief that the carcinogenic potential of many aromatic nitro compounds is probably caused by their biological reduction to hydroxylamino intermediates by the scheme:

$$RNO_2 \rightarrow RNO \rightarrow RNHOH \rightarrow RNH_2$$

These intermediates would presumably share the common properties of all carcinogens as suggested by Miller and Miller (1971) and Kriek (1974), in that they 1) are generated in their active form by reaction with intracellular macromolecules at the tissue site where they initiate carcinogenesis, 2) are transient intermediates if formed by metabolic interconversion, 3) are highly electrophilic, and 4) are all strong mutagens.

Cancer formation in both animals and man induced by the aromatic amines has been known for many years and was recently reviewed by several authors (Kriek, 1974; Arcos and Argus, 1974). The nitroaromatic compounds, on the other hand, have not received extensive treatment in the cancer literature, even though it is clear that their metabolism may involve many of the same active nitroso and hydroxylamino intermediates. Generally speaking, the nitro analogs of aromatic amine carcinogens, however, will be considerably less active as tumor-producers. In all likelihood this is due to the fact that they must first be enzymatically reduced to active forms, a process which is species-dependent and may occur very slowly in vivo.

In terms of structure-activity relationships among aromatic carcinogens, a fundamental observation is that unsubstituted polycyclic aromatic hydrocarbons with less than four condensed rings do not generally possess any carcinogenic activity (Arcos and Argus, 1974). The addition of amino groups or other so-called "amine-generating" groups (nitro, nitroso, hydroxylamino) to the inactive polycyclic nucleus can produce significant carcinogenic activity. The most important aromatic skeletons which become carcinogens by amino substitution are pictured in Figure 71. For maximum carcinogenic activity, the amino substituent(s) must be introduced at the terminal carbon atom(s) of the longest conjugated chain, as illustrated in Figure 71.

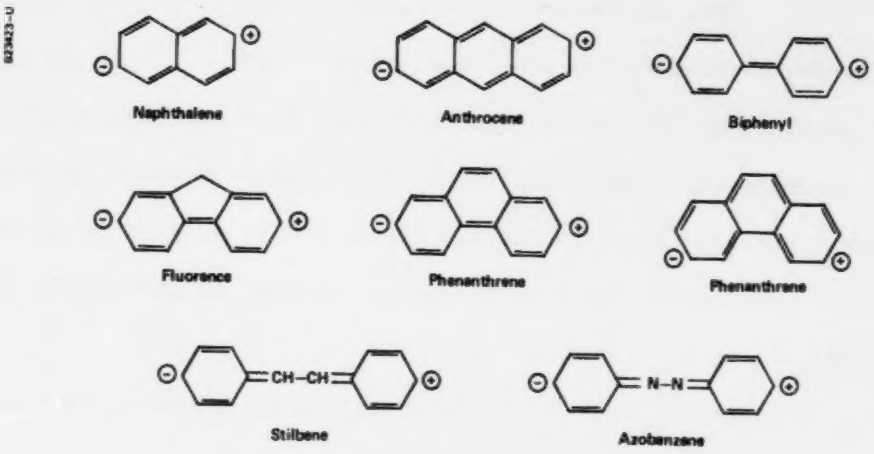


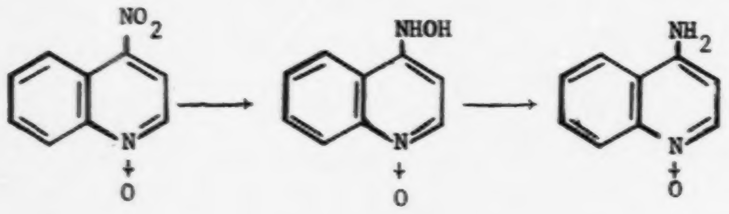
Figure 71. Polycyclic Aromatic Carcinogen Skeletons (Arcos and Argus, 1974)

Several nitro derivatives of the above structures have already been identified as tumorigens in animals. However, many of the nitroaromatic compounds have yet to be tested for carcinogenicity, while others have been found to be inactive as tumor-producing agents. Several of the nitroaromatic chemicals which have been tested for carcinogenicity are presented in Table 116. A discussion follows concerning individual nitroaromatic compounds of particular interest to the cancer problem.

a. 4-Nitroquinoline-N-oxide (4NQO)

The carcinogenic properties of 4NQO have been the subject of many intensive studies in the past (Endo *et al.*, 1971). It is one of the more potent carcinogens used in experimental tumor studies, causing cancer at the site of application in cutaneous or subcutaneous tissues. In addition, 4NQO can cause lung carcinomas in the rat (Mori, 1962), as well as increased hepatoma occurrence in rats fed dimethylaminoazobenzene as part of the diet (Takayama, 1961).

While 4NQO is predominantly an experimental tool and not an industrial compound with environmental contamination potential, it is important nevertheless to consider the biological fate of this classic nitroaromatic carcinogen and its relationship to the metabolism of nitroaromatics in general. Sugimura *et al.* (1966) studied the activity of an enzyme in rat liver and lung that reduces 4NQO to 4-hydroxyaminoquinoline-N-oxide (4HAQO) by the following scheme:



4NQO 4HAQO

Figure 72. Metabolic Conversions of 4NQO (Sugimura *et al.*, 1966)

Table 116. Nitroaromatic Compounds Tested for Carcinogenicity

Compound	Species	Strain or Type	Number and Sex	Preparation and Administration	Duration of Experiment	Effects	Reference
2-Amino-4-(p-nitrophenyl)thiazole	Rats	Sprague-Dawley	35 (F)	.01% in the diet for weeks 0-6; then .025% for weeks 6-20; then .050% for weeks 20-21; then .010% for weeks 21-25	50 weeks	23 of 35 rats developed adenocarcinoma of the breast	Cohen et al., 1975
1-Chloro-2,4-dinitronaphthalene	Rats	Sprague-Dawley	20 (F)	500 mg oral suspension-single dose	6 months	Of 19 survivors, 2 developed breast cancer, 1 developed adenocarcinoma of the lung	Griswold et al., 1966
	Rats	Sprague-Dawley	20 (F)	10 doses every 3 days for 30 days by gastric intubation (total dose = 3,000 mg)	9 months	Of 17 survivors, 2 had carcinoma, 2 had fibroadenoma and 1 had hyperplasia of the breast	Griswold et al., 1968
2,6-Dichloro-4-nitroaniline	Rats	Fischer	3 (M) + 2 (F)	100 mg orally 5 times a week for 52 weeks	18 months	One male rat developed a testicular interstitial cell tumor	Hadidian et al., 1968
	Rats	Fischer	14 (M) + 15 (F)	30 mg orally 5 times a week for 52 weeks	18 months	Six males developed testicular interstitial cell tumors; one female developed an adenocarcinoma of the breast	Hadidian et al., 1968
	Rats	Fischer	12 (M) + 12 (F)	0.3-10 mg orally 5 times a week for 52 weeks	18 months	Six males developed testicular interstitial cell tumors, one had a thyroid adenoma and one had a fibroma; one female developed a lymphoma	Hadidian et al., 1968
2,4-Dinitroanisole	Rabbits	?	?	3% in propylene glycol daily on the skin	90 days	No tumors produced	Draize et al., 1948

Table 116. Nitroaromatic Compounds Tested for Carcinogenicity (Cont'd)

Compound	Species	Strain or Type	Number and Sex	Preparation and Administration	Duration of Experiment	Effects	Reference
4,6-Dinitro-o-cresol	Rabbits	White	?	5% in olive oil, 20 applications to the skin	4 weeks	No tumors produced	Spencer et al., 1948
	Rats	Dow and B & L	102 (?)	0.002-0.05% in the diet	182 weeks	No tumors produced	Spencer et al., 1948
	Rats	Albino	?	200 ppm in the diet (15 mg/kg/day)	18 weeks	No tumors produced	Parker et al., 1951
3,4-Dinitro-dimethylaniline	Rats	Holtzman	10 (M)	2.67 mM/kg of diet for 12 months	12 months	No tumors produced	Miller et al., 1957
2,5-Dinitro-fluorene	Rats	Holtzman	10 (M)	1.62 mM/kg of diet for 8 months	10 months	One female developed mammary cancer; one female had a fibroadenoma of the breast	Miller et al., 1962
2,7-Dinitro-fluorene	Rats	Holtzman	8 (M) + 8 (F)	1.62 mM/kg of diet for 8 months	10 months	Eight females and one male developed mammary cancer; two rats had cancer of the small intestine	Miller et al., 1962
2,4-Dinitrophenol	Rats	Dow and B & L	70 (M)	0.01-0.10% in the diet	179 days	No tumors produced	Spencer et al., 1948
1,2,3,4,5,6-Hexachloro-7-nitronaphthalene	Rats	Sprague-Dawley	20 (F)	500 mg oral suspension as a single dose	6 months	Of 19 survivors, none developed tumors	Griswold et al., 1966
2,2',4,4',6,6'-Hexanitrostilbene	Rats	Sprague-Dawley	20 (F)	10 doses every 3 days by gastric intubation (total dose = 800 mg)	9 months	All animals survived, no tumors were produced	Griswold et al., 1968

Table 116. Nitroaromatic Compounds Tested for Carcinogenicity (Cont'd)

Compound	Species	Strain or Type	Number and Sex	Preparation and Administration	Duration of Experiment	Effects	Reference
2-Hydrazino-4-(p-nitrophenyl)-thiazole	Rats	Sprague-Dawley	35 (F)	0.10% in the diet for weeks 0-46; (total dose = 540 mg/rat)	75 weeks	29 rats developed tumors; 11 were adenocarcinomas and 9 were fibroadenomas of the breast	Cohen et al., 1973
N-Methyl-N-2,4,6-tetranitroaniline (tetryl)	Rats	Sprague-Dawley	20 (F)	10 doses every 3 days for 30 days by gastric intubation (total dose = 400 mg)	9 months	Of 19 survivors, 1 had hyperplasia of the breast, 1 had an adenoma of the stomach	Griswold et al., 1968
p-Nitrobiphenyl	Dogs	Mongrel	4 (F)	300 mg orally 3 times a week	33 months	2 dogs developed urinary bladder cystic carcinomas at 25 and 33 months; 1 dog developed invasive cystic epithelial tumor masses of the bladder at 33 months	Deichmann et al., 1965
	Dogs	Mongrel	4 (F)	300 mg orally each day	33 months	3 of 4 dogs developed urinary bladder cystic squamous cell carcinomas	Coplan, 1960
2-Nitrofluorene	Rats	Holtzman	20 (M)	1.62 mM/kg of diet	12 months	17 rats had squamous cell carcinomas of the forestomach; 13, 4, 2, and 1 had tumors of the liver, ear duct, small intestine, and breast, respectively	Miller et al., 1955
5-Nitro-2-furaldehyde semicarbazone	Rats	Sprague-Dawley	20 (F)	10 oral doses every 3 days for 30 days (total dose = 500 mg)	9 months	Of 5 survivors, 1 had carcinoma of the breast	Griswold, 1968
	Rats	Sprague-Dawley	10 (F)	10 oral doses every 3 days for 30 days (total dose = 350 mg)	9 months	All animals survived; 1 had hyperplasia of the breast	Griswold, 1968

Table 116. Nitroaromatic Compounds Tested for Carcinogenicity (Cont'd)

Compound	Species	Strain or Type	Number and Sex	Preparation and Administration	Duration of Experiment	Effects	Reference
N-[4-(5-Nitro-2-furyl)-2-thiazolyl] acetamide	Rats	Sprague-Dawley	35 (F)	0.199% in the diet for 46 weeks (total dose = 10.3 g/rat)	66 weeks	Of 34 survivors, 20 had fibroadenoma of the breast	Cohen et al., 1975
2-Nitronaphthalene	Monkey	Rhesus	1 (?)	242 mg/kg by stomach tube 6 days a week	54 months	Multiple papillary tumors and 1 fungating tumor of the bladder	Conzelman et al., 1970
p-Nitroperbenzoic acid	Mice	ICR/Ha Swiss Millerton	15 (F)	0.05 mg given s.c. once weekly for 26 weeks	21 months	2 mice developed sarcomas at the injection site	VanDuuren and Katz, 1972
	Mice	ICR/Ha Swiss Millerton	16 (F)	1 mg given s.c. once weekly for 26 weeks	21 months	No tumors observed; 9 of 16 mice had died by 6 months	VanDuuren and Katz, 1972
	Mice	CFN (Swiss Webster)		0.05 mg given s.c. once weekly for 26 weeks	21 months	1 mouse developed a sarcoma at the injection site; 4 other tumors were also observed	VanDuuren and Katz, 1972
o-Nitrophenol	Mice	Sutter	31 (F)	20% solution in dioxane given twice weekly on the skin for 12 weeks	12 weeks	Among 30 survivors, no tumors were seen	Boutwell and Bosch, 1959
p-Nitrophenol	Mice	Sutter	31 (F)	20% solution in dioxane given twice weekly on the skin for 12 weeks	12 weeks	Among 30 survivors, no tumors were seen	Boutwell and Bosch, 1959
p-Nitrostilbene	Rats	BDI and Wistar	27 (F)	0.01%; then 0.04% in the diet	Over 400 days	11 rats had papillomas and forestomach squamous cell carcinomas; 2 had otic carcinomas	Druckrey et al., 1955

Since it has been shown that 4HAQO is a more potent carcinogen than 4NQO itself (Endo and Kume, 1965; Shirasu, 1965) it was suggested that 4NQO is carcinogenic by virtue of its reduction to 4HAQO. Furthermore, its specific carcinogenic activity in the lung and liver, the sites where a 4NQO-reducing enzyme has been located, supports the argument that hydroxylamine derivatives of nitroaromatic compounds are the true proximate carcinogens involved in tumor-production.

b. Nitrobenzene Derivatives

Several of the polychlorinated nitrobenzenes are active both as tumor initiators and complete carcinogens. Searle (1966 a) treated the skin of male and female mice twice weekly with 0.2 ml of a 0.3% solution in acetone of pentachloronitrobenzene (PCNB) to test its tumor-initiating ability. He also tested 2,3,4,5-, 2,3,4,6-, and 2,3,5,6-tetrachloronitrobenzene (TCNB) under similar conditions. After 12 weeks of treatment, all mice received applications of croton oil, a well-known tumor-promoting agent, for 20 weeks (a tumor-initiating substance is one that produces tumors or carcinomas only after the subsequent application of a tumor-promoting chemical such as croton oil or phorbol ester). The results clearly indicated that all of the substances tested were active tumor-initiators (Table 117, Figure 73). Multiple skin papillomas began to show in all groups after five to eight weeks of croton oil treatment and progressed until five to ten weeks after cessation of treatment, whereupon some tumors regressed. The males in this experiment clearly showed a tendency to develop tumors earlier and in greater number than did females, except in the case of PCNB, where the reverse was true. This apparent sex difference in tumor development could not be explained by the results of the study.

Table 117. Incidence of Skin Tumors on Mice During and After Treatment with Croton Oil, Following Applications of Some Chloromononitrobenzenes[a] (Searle, 1966 a)

TEST COMPOUND	NO & SEX	SURVIVORS AT[a]				MICE WITH TUMORS AT[a]				TOTAL TUMORS AT				WK[a] TO 50% TUMOR INCIDENCE
		10 WK	20 WK[b]	30 WK	40 WK	10 WK	20 WK[b]	30 WK	40 WK	10 WK	20 WK[b]	30 WK	40 WK	
Acetone controls	(15)M	10	9	8	7	1	6	5	1	1	7	6	3	16
	(15)F	10	10	8	7	0	3	4	4	0	5	7	7	
Pentachloronitrobenzene	(15)M	10	10	9	8	2	6	8	7	4	23	29	26	13
	(15)F	10	9	7	4	6	7	7	7	13	27	33	28	10
2,3,4,5-Tetrachloronitrobenzene	(15)M	8	7	6	5	6	7	7	7	26	54	61	57	7
	(15)F	8	5	3	3	4	7	7	7	5	14	19	22	14
2,3,4,6-Tetrachloronitrobenzene	(15)M	10	8	7	6	4	6	6	6	12	31	39	37	13
	(15)F	10	7	4	1	3	5	5	5	4	10	11	11	19
2,3,5,6-Tetrachloronitrobenzene	(15)M	10	8	7	6	7	8	7	7	18	41	39	36	8
	(15)F	10	8	6	5	3	6	5	5	6	13	14	11	15

[a] Time is measured from start of croton oil treatment

[b] End of croton oil treatment

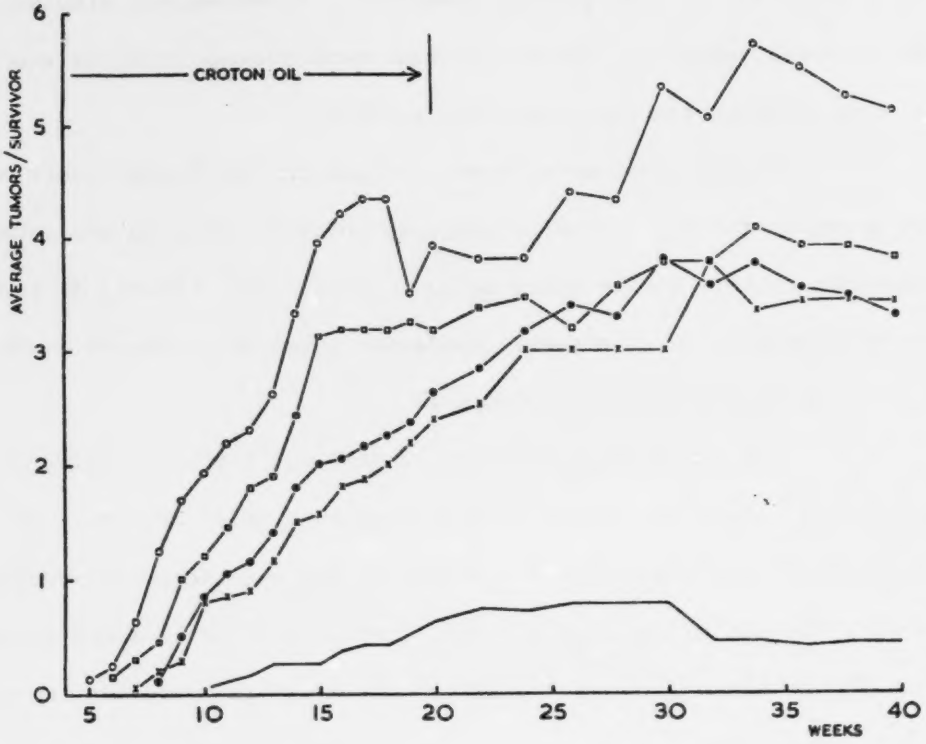


Figure 73. Average Number of Skin Tumors/Surviving Mouse During and After Treatment with Croton Oil (Searle, 1966 a)

KEY: Previous treatment with: acetone (————)
PCNB (●——●)
2,3,4,5-TCNB (○——○)
2,3,4,6-TCNB (X——X)
2,3,5,6-TCNB (□——□)

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Histologic examination of the papillomas formed revealed that the tumors were generally non-malignant, the exception being a single squamous-cell carcinoma on a mouse treated with PCNB, and one basal-cell carcinoma on a mouse due to 2,3,5,6-TCNB treatment. In subsequent studies, Searle (1966 b) established that these compounds were indeed active as complete carcinogens when administered subcutaneously to mice.

Searle (1966 a) pointed out that the metabolic reduction of the nitro group to the hydroxylamine has been shown to occur in the rabbit with all compounds in this series (Bray et al., 1957). This process is probably an important mechanism in skin tumor initiation, just as it may be in the activation of other nitroaromatic carcinogens.

The tumorigenic activity of PCNB was further studied by Innes et al. (1969). Male and female mice were given a daily oral dose of 464 mg/kg body weight of PCNB from 7 to 28 days of age and thereafter received the compound at 1206 ppm in the diet for the remainder of the 18 month study. Their results (Table 118) showed that PCNB can act alone in producing tumors at several different sites.

Strict pathological interpretation of the individual tumors encountered was not attempted in this investigation. For the most part, however, pulmonary tumors consisted mainly of adenomas, and lymphomas were usually Type B reticulum-cell sarcomas. The use of the term "hepatoma" in reporting results was not meant to imply that these tumors were benign, however. The authors felt that a distinction between malignant and benign liver tumors in the mouse could not be made, but that most hepatomas had malignant potential.

Table 118. Tumor Formation in Male and Female Mice Receiving PCNB (Innes _et_ _al_., 1969)

Compound	Strain*	Number of Mice at Term		Total Mice Necropsied		Weeks at Term		Mice with Hepatomas		Mice with Pulmonary Tumors		Mice with Lymphomas		Total Mice with Tumors	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
PCNB	X	14	18	18	18	78	78	2	4	2	1	2	0	5	5
	Y	16	17	17	17	78	78	10	1	1	0	1	1	11	2

*Strain X = $(C57BL/6 \times C3H/Anf)F_1$; strain Y = $(C57BL/6 \times AKR)F_1$.

Another nitrobenzene derivative, 1-fluoro-2,4-dinitrobenzene (DNFB), was found to be a powerful tumor promoter in female mice (Bock et al., 1969). Well-known as a potent skin-sensitizer (see Section III-D-3), DNFB was tested both as a tumor initiator and as a tumor-promoter in separate mouse skin-painting studies. Animals were painted five times a week for 32 weeks with 0.25 ml of acetone containing DNFB in concentrations ranging from 0.03% to 3.0%. Twenty-one days before DNFB treatment, selected groups of mice had been exposed to 125 μg of 7,12-dimethylbenzanthracene (DMBA) as a tumor-initiating stimulus. Another group received DMBA followed by painting with 0.03% croton oil and served as positive controls. The results presented in Tables 119 and 120 show that DNFB, while inactive as a tumor-initiator, was a very effective tumor-promoting agent. The first skin tumors produced by DNFB appeared after four weeks of treatment, as compared to the croton oil-treated positive control group, where tumors first appeared after six weeks. By the end of the experiment, however, a larger number of tumors had been produced by the croton oil treatment.

The authors concluded that, aside from the phorbol esters (the active agents of croton oil), DNFB is one of the most potent tumor-promoting agents known. While the mechanism of this observed tumor-promoting activity is unknown, it has been postulated that a relationship may exist between tumor promotion and disturbance of the immune system. The powerful ability of DNFB and other skin-sensitizers (e.g., 1-chloro-2,4-dinitrobenzene) to form nonspecific conjugates with amino acids, peptides, or proteins in the body may be important in the expression of this tumorigenic activity. Evidence has not been encountered, however, which demonstrates any tumor-producing capabilities for other nitroaromatic skin-sensitizers, such as 1-chloro-2,4-dinitrobenzene.

Table 119. Tumor Promotion in Female Mice by 1-Fluoro-2,4-dinitrobenzene (DNFB) (Bock *et al.*, 1969)

Initiating Stimulus	Promoting Stimulus[a]	No. of Mice at Risk[b]	Mice with Tumors		Total No. of Tumors	No. of Mice in Which Tumors Regressed
			No.	%		
None	0.03% croton oil	56	1	2	1	1
2 X 2500 μg DNFB	0.03% croton oil	38[d]	1	3	1	1
125 μg DMBA	None	30	0	0	0	0
	0.03% croton oil	30	15	50	79	0
	0.3% DNFB[c]	24	6	25	8	2
	0.1% DNFB	30	21	70	41	1
	0.03% DNFB	21	9	38	11	0

[a] 0.25 ml of acetone solution 5 times a week for 32 weeks.

[b] ICR Swiss mice surviving at least 2 weeks of promoting stimulus.

[c] 6 of 30 mice died after only 8 applications of 0.3% DNFB; further treatment was discontinued.

[d] 22 of 60 mice died after the second DNFB treatment.

Table 120. Tumor Promotion by 1-Fluoro-2,4-dinitrobenzene (DNFB) in Various Stocks of Female Mice (Bock et al., 1969)

Strain of Mouse	Promoting Stimulus[a]	Duration of Promotion (weeks)	No. of Mice at Risk	Mice with Tumors No.	Mice with Tumors %	Total No. of Tumors	Tumors per Tumor-bearing Mouse
Swiss	0.1% DNFB[b]	50	50	35	70	55	1.6
C57BL/6		50	30	6	20	8	1.3
BALB/c		14	30	5	17	7	1.4
Swiss	Acetone only	50	50	2	4	2	1.0
C57BL/6		50	30	0	0	0	
BALB/c		14	30	0	0	0	
Swiss	0.03% croton oil	50	50	49	98	346	7.1
C57BL/6		50	30	20	67	35	1.8
BALB/c		14	30	5	17	8	1.6
Swiss	0.5% dinitrophenyllysine hydrochloride	34	50	0	0	0	

[a] 0.25 ml of acetone solutions, applied 5 times a week beginning 21 days after a single application of 125 μg of DMBA in 0.25 ml of acetone.

[b] 50 Swiss mice treated with 0.1% DNFB, but not with DMBA, developed no tumors in 38 weeks.

c. Heterocyclic Nitro Compounds

Several nitrofuran and nitroimidazole compounds have been synthesized as anitbacterial agents and are widely used as feed additives in animal meat production. Studies by Cohen et al. (1973, 1975) demonstrated that several nitrofuran and nitroimidazole compounds were effective carcinogens (Table 116).

d. Nitro Derivatives of Aromatic Amine Carcinogens

Recently, a number of nitro derivatives of naphthalene, biphenyl, and fluorene have been shown to produce cancer in laboratory animals. It is well-known that many of the amino derivatives of these same aromatic structures are proven carcinogens in humans as well as animals.

Para-aminobiphenyl (xenylamine) is a powerful bladder carcinogen in humans (Arcos and Argus, 1974; Kriek, 1974) whose activity has been recognized for a number of years. A study by Deichmann et al. (1965) has indicated that para-nitrobiphenyl (PNB), an intermediate in the manufacture of para-aminobiphenyl, is just as potent a carcinogen in dogs as the amino derivative. Furthermore, they state that the bladder tumors found in humans who were occupationally exposed to para-aminobiphenyl may well have been partially caused by PNB. Their data indicated that 7 to 10 g/kg body weight of PNB fed to dogs over a period of 25 to 33 months produced tumors of the bladder (Table 116). This compared with 8.2 to 14.1 g/kg body weight of para-aminobiphenyl fed over a period of 29 to 33 months to produce similar tumors. Obviously, both compounds were about equal in their tumorigenic potency.

In another investigation, Coplan (1960) compared the pathology of PNB-induced bladder tumors in dogs with those resulting from para-aminobiphenyl (Table 116). The dog was chosen as the animal model to study because of the close similarity in the histological and functional structure of the urinary bladder in both man and dog. It was found that the bladder tumors induced by the feeding of either compound were always medium-grade squamous-cell carcinomas and identical in every respect. From the above evidence, PNB must be regarded as carcinogenic to humans and handled with the same precautions accorded to para-aminobiphenyl.

Another agent known to produce bladder tumors among men in the dyestuff industry is 4,4'-diaminobiphenyl (benzidine). The carcinogenicity of benzidine has been extensively reviewed by several authors (Hueper, 1969; Scott, 1962; Arcos and Argus, 1974). A report has also been made by Laham et al. (1964) stating that the closely related compound 4,4'-dinitrobiphenyl is similarly carcinogenic to laboratory animals.

2-Acetylaminofluorene is a potent carcinogen over a wide range of tissues and species when administered systemically (Kriek, 1974). The addition of nitro groups to the fluorene molecule also produces compounds with carcinogenic activity (Miller et al., 1955). The feeding of 2-nitrofluorene to rats for a period of eight months produced a high incidence of multiple tumors in the forestomach, particularly squamous-cell carcinomas (Table 116). This carcinogenic effect of 2-nitrofluorene was particularly interesting due to the high incidence of cancers (17 of 18 rats) and the unusual specificity for gastric tumor formation. The stomach is a site where most carcinogens generally do not produce malignancies.

Another derivative of fluorene which possesses carcinogenic activity is 2,7-dinitrofluorene. The activity of this compound was studied by Miller et al. (1962) along with that of 2,5-dinitrofluorene. Only the 2,7-isomer exhibited strong carcinogenic properties when fed to rats. These effects were manifested mainly as mammary cancers (Table 116). In tissues other than the liver, 2,7-dinitrofluorene was found to be equally carcinogenic as 2-acetylaminofluorene.

Bladder cancer in man has also been reported to be caused by exposure to 2-naphthylamine (Arcos and Argus, 1974). Several nitro analogs of this potent carcinogen have likewise exhibited tumorigenic activity in animals. One of these compounds, 2-nitronaphthalene, has been reported to induce numerous benign papillomas in the urinary bladder of a Rhesus monkey when it was fed for almost five years (Conzelman et al., 1970, Table 116). On the basis of these findings, the author suggested that the same precautions be observed when handling 2-nitronaphthalene as with 2-naphthylamine or 4-nitrobiphenyl. Treon and Cleveland (1960) were unable to induce tumors in either of two monkeys by the single dose feeding of 2.1 or 4.7 gm/kg of 2-nitronaphthalene.

A nitro derivative closely related to 2-naphthylamine is 3-nitro-2-naphthylamine, which was tested for carcinogenicity in rats by Weisburger et al. (1967). The compound was administered by oral lavage five times weekly for 52 weeks at a dose of 10 mg per day (one-third the maximally tolerated level). Twelve tumors of the forestomach developed in 8 of 60 male rats after 555 days, while 7 of 60 females developed a total of 12 tumors after 420 days; 4 lesions of the forestomach, 6 of the mammary gland, and 2 at other sites.

Weisburger and coworkers (1967) also tested 1,2-dichloro-3-nitronaphthalene for carcinogenicity in rats and found it to be less active than 3-nitro-2-naphthylamine. After treatment with 30 mg five times weekly for 52 weeks, 3 of 60 males and 5 of 60 females developed tumors by the end of the experiment (564 days). The males developed two fibromas and one pituitary adenoma, while four of the five females developed mammary tumors.

A more detailed examination of the carcinogenicity of 1,2-dichloro-3-nitronaphthalene at different dose levels in rats was made by

Hadidian et al. (1968). As in the above study, the compound was found to be only weakly tumorigenic when compared to controls (Table 121). Comparison of 1,2-dichloro-3-nitronaphthalene with 3-nitro-2-naphthylamine again found the latter compound to be a much more active carcinogen and, in fact, more potent than 2-naphthylamine under the conditions employed. As was seen with the case of 2-nitrofluorene, an unusual propensity for the induction of forestomach tumors was exhibited by 3-nitro-2-naphthylamine (Table 121).

Another naphthalene derivative, 1-chloro-2,4-dinitronaphthalene, has shown weak carcinogenic activity in animals. Griswold et al. (1966) studied the compound because it has a nitro group in the important 2-position of naphthalene and is used for various insecticide, fungicide, and synthesis applications. Administration of a single 500 mg/kg body weight dose by gastric intubation in female rats produced carcinomas in three of twenty animals. Two lobular carcinomas of the breast and one adenocarcinoma of the lung were observed (Table 116).

In a subsequent study, the effects of multiple intragastric doses of 1-chloro-2,4-dinitronaphthalene were tested for carcinogenicity (Griswold et al., 1968). The dosages were selected on the basis of preliminary determinations to find the maximally tolerated dose. A total of 3,000 mg of 1-chloro-2,4-dinitronaphthalene was administered to each of 20 rats over a period of just under 45 days. Of the 17 survivors of the acute and chronic effects from exposure to the chemical, two developed carcinomas of the breast and two developed fibroadenomas (Table 116). The high total dosage of compound administered in this study to produce two malignant tumors indicates that 1-chloro-2,4-dinitronaphthalene is probably not one of the more potent carcinogenic threats to man.

Table 121. Carcinogenesis by 1,2-Dichloro-3-nitronaphthalene and 3-Nitro-2-naphthylamine (Hadidian _et al._, 1968)

Compound[a]	Number and Sex	Dose (mg)	Neoplastic Growths, Number of Rats with Tumors	Non-Neoplastic Lesions, Number of Rats with Lesions
3-Nitro-2-naphthylamine	3 M	30	Interstitial cell tumor - testis, 1 Papilloma - stomach, 2 Squamous cell carcinoma (ear, stomach), 2 Adenoma (pituitary, lung), 2 Adenocarcinoma - breast, 1	Hepatotoxicity, 1
	3 F	30	Papilloma - stomach, 2 Adenocarcinoma - breast, 2 Squamous cell carcinoma - ear, 1 Metastasis, 1	Hepatotoxicity, 1
	15 M	10	Interstitial cell tumor - testis, 10 Papilloma - stomach, 8 Lymphoma, 2 Mesothelioma - testis, 1 Adenoma - adrenal, 1 Metastasis, 2	Hepatotoxicity, 1
	15 F	10	Adenocarcinoma - breast, 5 Papilloma - stomach, 4 Fibroadenoma - breast, 2 Fibrosarcoma - cervix, 1	Hepatotoxicity, 1 Hyperplasia - endometrium, 1 Polyp - uterus, 1
	3 M	3	Interstitial cell tumor - testis, 2 Papilloma - stomach, 1	
	3 F	3	Fibroadenoma - breast, 2 Papilloma - stomach, 3	
	3 M	100	Interstitial cell tumor - testis, 1 Papilloma - stomach, 1 Basal cell carcinoma - stomach, 1	
	3 F	10	Papilloma - stomach, 2	
	3 M	0.3	Interstitial cell tumor - testis, 3	
	3 F	0.3	Papilloma - stomach, 1 Lymphoma, 1	

[a] Compounds were administered by gastric intubation, in a volume of 1.0 ml or less, 5 days per week for 52 weeks. Observation of animals continued for 6 months past the end of treatment (total duration of study = 18 months).

Table 121. Carcinogenesis by 1,2-Dichloro-3-nitronaphthalene and 3-Nitro-2-naphthylamine (Hadidian *et al.*, 1968) (Cont'd)

Compound[a]	Number and Sex	Dose (mg)	Neoplastic Growths, Number of Rats with Tumors	Non-Neoplastic Lesions, Number of Rats with Lesions
1,2-Dichloro-3-nitronaphthalene	3 M	100	Interstitial cell tumor - testis, 2 Adenocarcinoma - lung, 1 Fibroadenoma - breast, 1 Mesothelioma - testis, 1	
	3 F	100	None	Hyperplasia - endometrium, 1
	14 M	30	Interstitial cell tumor - testis, 7 Fibroma, 2 Adenoma - pituitary, 1	Hepatotoxicity, 2
	15 F	30	Fibroadenoma - breast, 4 Fibrosarcoma, 1	Hyperplasia - breast, 1 Polyp - uterus, 1
	3 M	10	Interstitial cell tumor - testis, 2	
	3 F	10	Fibroadenoma - breast, 2	
	3 M	3	Interstitial cell tumor - testis, 3	
	3 F	3	None	
	3 M	1	Interstitial cell tumor - testis, 1	
	3 F	1	None	Polyp - uterus, 1
	3 M	0.3	Interstitial cell tumor - testis, 2	
	2 F	0.3	None	

[a] Compounds were administered by gastric intubation, in a volume of 1.0 ml or less, 5 days per week for 52 weeks. Observation of animals continued for 6 months past the end of treatment (total duration of study = 18 months).

e. Miscellaneous Nitroaromatic Carcinogens

A carcinogenic bioassay of *p*-nitroperbenzoic acid was recently conducted in two laboratories and reported by Van Duuren *et al*. (1972). Mice were injected subcutaneously once weekly for 26 weeks and observed for subsequent tumor development. In one laboratory, *p*-nitroperbenzoic acid at 0.05 mg injected subcutaneously once weekly for 26 weeks (total dose, 1.3 mg) produced two sarcomas at the injection site in a group of 15 mice. In a separate study, the same dose produced one sarcoma and four tumors at other sites in a group of 16 animals (Table 116).

Recent concern over the toxic properties of certain hair dye constituents has led to investigations of their long-term adverse effects, including carcinogenicity. Two hair dyes have now been tested, one of which contains 2-nitro-*p*-phenylenediamine (2-NPPD) and 4-nitro-*o*-phenylenediamine (4-NOPD), and the other containing an aminonitrophenol (Searle *et al*., 1975). Commercial hair dye preparations containing these substances were tested by repeated topical applications in aqueous acetone solutions to the skin of mice. These tests were intended to resemble actual patterns of human usage. Preliminary results demonstrated the formation of malignancies in 5 out of 48 mice receiving the preparation containing the two isomers of nitrophenylenediamine. A second strain of mice developed cancers in 4 out of 52 animals receiving the same preparation. Two out of 32 mice of one strain and 3 out of 32 mice of a different type developed malignant tumors when exposed to the hair dye component containing aminonitrophenol.

All tumors in these experiments were of the lymphoid system; no tumors were seen on the treated skin. Three mice developed lymphosarcomas, while the rest had malignant lymphomas involving the spleen, with infiltration to the liver and other organs. The possibility of oral ingestion of the compound by mice during the process of grooming must be considered here, as well as the potential role of oncogenic viruses which are known to cause lymphomas in mice. Since these results are preliminary, a final judgement regarding the carcinogenic hazard of certain hair dyes must be reserved until more extensive tests have been completed.

An observation was made by van Esch and coworkers (1957) that rats being fed for long periods with hexanitrodiphenylamine developed large multiple mammary tumors. In a subsequent study they treated female rats with hexanitrodiphenylamine in the diet at 500 ppm for about two and one-half years. In addition, they also fed groups of rats with the closely related compounds picramide and picric acid at the same concentration.

Their results, presented in Table 122, show a high incidence of tumor formation in all treated groups. In the control animals, however,

Table 122. Incidence of Mammary Tumors in Female Rats Treated with Hexanitrodiphenylamine (van Esch et al., 1957)[a]

Group	No. of Rats	No. Alive After 2 Years	No. With Mammary Tumors	Average Age of Appearance of Tumors
Controls	19 (83)	12 (63)	8 (18)	25 months (26)
Hexanitrodiphenylamine	10	1	10	19 months
Picric acid	10	7	4	22 months
Picramide	8	7	4	29 months

[a] Dosage, 500 ppm mixed in dry food. The numbers in parentheses indicate total incidence as found in untreated rats, including that from other experiments during the same period.

a very high frequency of tumor development was also noted which tends to discount the results seen with picramide and picric acid. Hexanitrodiphenylamine, on the other hand, caused the formation of multiple mammary tumors (average, three per animal) in all treated rats, whereas control animals rarely developed more than a single tumor. All tumors in this experiment, including those in untreated rats, were non-malignant fibroadenomas, adenofibromas, or adenomas of the breast.

7. Possible Synergisms

Exposure to chemical substances often results from contact with a mixture of compounds rather than a single pure source. Even though the majority of occupational, domestic, and environmental exposures to nitroaromatic chemicals will probably involve the concomitant exposure to any number of synthetic organic substances, little attention has been given to the dangers of synergistic and joint toxic actions. A report has been made by Smyth et al. (1969) which characterized the relative synergistic toxic effects of nitrobenzene in combination with 26 industrial chemicals. These results are presented in Table 123.

Overall, nitrobenzene paired with various chemicals resulted in 11 pairs where the ratio of predicted/observed LD_{50} exceeded 1.00 and 14 pairs where the ratio was less than 1.00. The median unadjusted ratio was 0.98, which does not tend to indicate a high potential for synergistic toxicity under the conditions employed. While this relationship may hold true for other derivatives of nitrobenzene, these results should not be extrapolated to the dinitrophenols, which produce their toxic effects by an entirely different mechanism.

Table 123. Unadjusted Ratios of Predicted to Observed LD_{50} Values of Nitrobenzene Mixed by Volume with Various Chemicals (Smythe _et_ _al_., 1969)

Nitrobenzene Mixed With	Predicted/Observed LD_{50}
Acetone	1.47
Acetonitrile	0.85
Acetophenone	0.98
Acrylonitrile	0.82
Aniline	1.32
Butyl Cellosolve	0.85
Butyl Ether	1.28
Carbon Tetrachloride	1.47
Diethanolamine	0.70
Dioxane	1.39
Ethyl Acetate	0.97
Ethyl Acrylate	1.07
Ethyl Alcohol	0.97
Ethylene Glycol	1.07
Formalin	1.20
Isophorone	0.62
Morpholine	0.86
Phenyl Cellosolve	1.12
Polyethylene Glycol 200	0.72
Propylene Glycol	1.00
Propylene Oxide	0.87
Tergitol XD	0.92
Tetrachloroethylene	0.82
Toluene	0.78
Ucon 50HB260	0.99
Ucon LB250	1.26

In studying the affects of chronic poisoning in rats with m-dinitrobenzene, Baede and Kiese (1949) noted a marked synergism with ethanol. While dogs are not affected, chronically ethanol-poisoned rats are more susceptible to the effects of m-dinitrobenzene. Furthermore, in chronically m-dinitrobenzene-poisoned rats, ethanol has a more pronounced and long-lasting effect. In addition, propanol, isopropanol, and acetic acid, but not butanol, produced a similar synergism with m-dinitrobenzene.

E. Toxicity to Lower Animals

Several studies have been encountered which demonstrate selective toxicity for various species of fish and aquatic organisms by nitroaromatic compounds. The most prominent group of chemicals in this class which have been tested for toxicity are the mononitrophenols (metabolite of parathions), especially those containing halogens. Additional studies have also been conducted to determine the effects of nitroaromatic herbicides, nitrosalicylanilides, and TNT wastes on mortality among fish. Only very limited information is available concerning either the toxicity of non-phenolic nitroaromatics or the toxicity of nitroaromatic compounds to non-aquatic lower animals.

1. Nitrophenols

The problem of water-borne phenolic wastes has long been recognized as a serious threat to many species of fish (Blyth and Blyth, 1920). Lammering and Burbank (1960) conducted an investigation to look specifically at the adverse effects of o-nitrophenol on bluegill sunfish in order to determine the role of ortho-substitution of inorganic radicals on the phenol ring. Fish were maintained under standard static conditions and exposed to o-nitrophenol at various concentrations. Reactions of the fish to the substance were observed over a 48 hour period. Plots of survival versus time of exposure to o-nitrophenol at four different concentrations are shown in Figure 74.

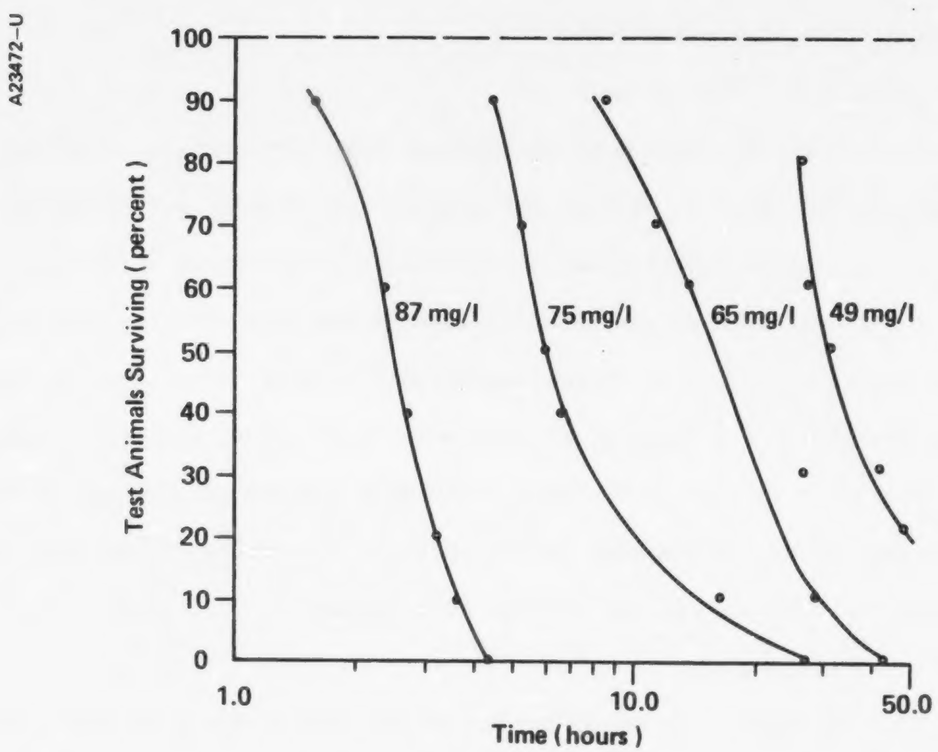


Figure 74. Mortality Curves for Various o-Nitrophenol Concentrations (Lammering and Burbank, 1960)

A loss of equilibrium occurred in fish at concentrations equal to or greater than 49 mg/l during the second 24 hour period. The median tolerance limit (concentration that kills 50 percent of the fish) for o-nitrophenol was determined to be 66.9 mg/l for a 24 hour period and 46.3-51.6 for a 48 hour period.

The difference between the 24 hour and 48 hour tolerance limits indicates that a certain degree of cumulative toxicity is possible in bluegills exposed to o-nitrophenol. These results are in contrast to those obtained by exposing fish to phenol or chlorophenol, where little difference was seen between 24 hour and 48 hour tolerance limits. Even though the acute toxicity of o-nitrophenol is three-fold less than for phenol, the possiblity of chronic poisoning by its cumulative action may create serious environmental problems.

The actual symptoms of poisoning in fish exposed to phenol, chlorophenol, and o-nitrophenol were quite similar in all cases. These included nervous twitching with continuous quivering of the fins and jaws, suggesting an increased rate of respiration. Rapid paroxysmal swimming was noted in response to slight movements near the fish tanks, indicating that the fish were very excitable. The loss of equilibrium preceded death by a considerable length of time and occurred either as a temporary partial loss or, more commonly, a permanent loss of function. These reactions of bluegills to phenolic contaminants were so characteristic as to be potentially useful as a qualitative means of bioassay for their detection.

2. Halogen-substituted Nitrophenols

Between 1953 and 1957 a total of 4,346 chemicals were screened for activity as selective toxicants in controlling the parasitic sea lamprey of the Great Lakes (Applegate *et al.*, 1966). From all these compounds, a single substance, 3-bromo-4-nitrophenol, was found to have the desired biological effect of being more toxic to sea lamprey larvae than to native fish species. This finding led to a series of exhaustive tests on halogen-substituted mononitrophenols for specific larvicidal activity. Results of these tests indicated that a small group of nitroaromatic chemicals possessed the desired property (Applegate *et al.*, 1967). These compounds all contained a nitro group in the 4-position on a phenolic nucleus and halogens or a trifluoromethyl group substituted directly on the ring.

Six compounds were initially identified as effective larvicides and tested further for toxicity to fish (Applegate *et al.*, 1958). The results of these acute toxicity tests under static conditions are presented in Table 124.

Table 124. Differential Toxic Effects Among Larval Lampreys and Fishes of Certain Mononitrophenols Containing Halogens (Applegate *et al.*, 1958) (Sodium salt is expressed in parts per million of free phenol.)

Name and Form of Compound	Concentration Required to Kill All Lamprey Larvae (ppm)	Concentration Required to Cause Significant Mortality † Among Fishes (ppm)		
		Rainbow trout	Brown trout	Bluegill sunfish
2-Bromo-4-nitrophenol				
Free phenol	5	13	11	
Na salt	7	15		
3-Bromo-4-nitrophenol				
Free phenol	5	11		15
5-Chloro-2-nitrophenol				
Free phenol	3	5	5	
2,5-Dichloro-4-nitrophenol				
Free phenol	3	13	7	
Na salt	5	17		
3,4,6-Trichloro-2-nitrophenol				
Free phenol	5	17	15	
Na salt	13	23		
3-Trifluoromethyl-4-nitrophenol[*]				
Free phenol	2	9	7	
Na salt	2	7	7	

[†] Mortality of approximately 10 percent of all test animals.

[*] α,α,α-Trifluoro-4-nitro-*m*-cresol

Under conditions that simulated an actual flowing stream, the lethal effects of the above substances on fish appeared to be lessened. Their effect on larval lampreys, however, remained essentially the same as in static tests.

Upon close examination using fish exposed to 3-trifluoromethyl-4-nitrophenol (TFM), death appeared to result from a complex of circulatory and respiratory failure (Applegate et al., 1966). Characteristic responses in the lamprey included respiratory edema, hemorrhage, and excess mucus secretion in the gill pouches. Severe vasodilation was observed, which was shortly followed by death. In rainbow trout TFM produced extreme anoxia. Also present were edema and vasodilation in the gills accompanied by excessive mucus. Death in the trout apparently resulted from anoxia produced by accumulation of mucus on the gill epithelium. Lamprey eel mortality, however, was more directly attributable to acute hypotension, with circulatory and cardiac failure. Differential susceptibility to nitroaromatic compounds between lampreys and fish was probably due to variation in the permeability of the respiratory epithelium to the chemical. It is also possible that sufficient interspecies variation exists in metabolic rates and pathways to significantly alter the half-life for elimination of the nitrophenols.

A more intensive study was undertaken (Applegate et al., 1966) on a number of related halogen-containing nitrophenols, after it was shown that this class of compounds may be useful in controlling the lamprey. Test animals included larvae of the sea lamprey (Petromyzon marinus L.) and fingerling rainbow trout (Salmo gairdneri). Each chemical was introduced into glass battery jars, holding four individuals of each species, at a maximum concentration of 40 ppm. Compounds which were found to be more toxic to lampreys than to fish were treated again using a total of 16 to 32 individuals of each species at varying concentrations. Table 125 summarizes the mortality data for 31 mononitrophenols containing either halogens or a trifluoromethyl group.

Table 125. Toxic Effects of 31 Mononitrophenols Containing Halogens or a Trifluoromethyl Group on Larval Lampreys and Fingerling Rainbow Trout (Applegate et al., 1966)

(All tests conducted for a 24 hour period at a water temperature of 55°F. No tests of compounds conducted at concentrations greater than 40 ppm. Abbreviations: Lampreys = larval lampreys; RBT = rainbow trout)

	Biological Activity				Properties of Compounds Collectively Toxic to Lampreys		
Compounds	No Toxic Effect on Either Lampreys or RBT	Equally Toxic to Lampreys and RBT	More Toxic to RBT than to Lampreys	More Toxic to Lampreys than to RBT	LD_{100}/24 hrs. for Lampreys (ppm)	LD_{25}/24 hrs. for RBT (ppm)	Differential Toxicity Expressed as Ratio of Concentrations
Derived from 2-nitrophenol:							
3-fluoro-			X		-	-	-
4-fluoro-	X				-	-	-
5-fluoro-	X				-	-	-
6-fluoro-	X				-	-	-
4,5-difluoro-			X		-	-	-
4,6-difluoro-			X		-	-	-
3-chloro-			X		-	-	-
4-chloro-			X		-	-	-
5-chloro-				X	3	5	1.7
4,5-dichloro-			X		-	-	-
4,6-dichloro-			X		-	-	-
3,4,6-trichloro-				X	5	17	3.4
4-bromo-			X		-	-	-
5-bromo-		X			-	-	-
4,6-dibromo-			X		-	-	-
3-trifluoromethyl-				X	<15	>40	2.7[†]
4-trifluoromethyl-			X		-	-	-
Derived from 3-nitrophenol:							
2-iodo-				X	>3	4	1.0[†]
Derived from 4-nitrophenol:							
2-fluoro-				X	17	23	1.4
3-fluoro-				X	12	17	1.4
2-chloro-				X	7	12	1.7
3-chloro-				X	4	10	2.5
2,5-dichloro-				X	3	13	4.3
2,6-dichloro-				X	?[†]	?[†]	?[†]
2,3,6-trichloro-			X		-	-	-
2,3,5,6-tetrachloro			X		-	-	-
2-bromo-				X	5	12	2.4
3-bromo-				X	5	11	2.2
2,6-dibromo-				X	19	25	1.3
2-trifluoromethyl				X	13	19	1.5
3-trifluoromethyl				X	2	9	4.5

[†] Tolerances poorly defined due to low solubility of compound.

All of the trifluoromethyl, monohalogen, and dihalogen derivatives of 4-nitrophenol in this study were significantly more toxic to lamprey larvae than to trout. The tri- and tetrahalogen-4-nitrophenols, on the other hand, were more toxic to the fish than to lampreys. Furthermore, these compounds were more toxic at lower concentrations than were any of the mono- and disubstituted 4-nitrophenols.

Moving the halogen from the 2- to the 3-position in the mononitrophenols did not greatly affect toxicity. The two isomers of trifluoromethyl-4-nitrophenol, however, were quite different in their species-specific and lethal effects. Placing the trifluoromethyl group in the 3-position of the ring increases the differential toxicity to 4.5 (see Table 125). 3-Trifluoromethyl-4-nitrophenol is now widely used in the Great Lakes for sea lamprey control.

Additional studies were carried out on unsubstituted mononitrophenols, halogenated di- and trinitrophenols, and one polyhalogenated nitrophenol (Applegate et al., 1966, 1967). Table 126 presents the results of tests where fish and lampreys were exposed to these chemicals at concentrations up to 5 ppm.

Earlier investigations had determined that aniline and nitroanilines were generally not toxic to fish unless a halogen was present. Similarly, the alkylated phenols, such as the cresols, were not highly toxic when a nitro group was added. On the other hand, the dinitroalkylphenols (e.g., dinitrocresol) were quite toxic to both fish and lampreys. In addition, the alkylated phenols containing both nitro groups and a halogen were also toxic.

Several investigators have reported that fish are unable to conjugate "foreign phenols" (Maickel et al., 1958, 1959) and, in fact, that

Table 126. Biological Activity of Phenol and Some Substituted Phenolic Compounds Other Than Mononitrophenols Containing Other Halogens[a,b] (Applegate *et al.*, 1966)

Compound	Biological Activity
Phenol	
Phenol (liquified USP XIV)	Toxic to RBT; not toxic to BG & L
Mononitrophenols	
x-nitrophenol	No toxic effect on any species
2-nitrophenol	ditto
3-nitrophenol	ditto
4-nitrophenol	ditto
Dinitrophenols	
2,4-dinitrophenol	Not toxic to L
Halo-dinitrophenols	
4-fluoro-2,6-dinitrophenol (40 ppm)	Not toxic to RBT & L
4-chloro-2,6-dinitrophenol (40 ppm)	ditto
2-chloro-4,6-dinitrophenol	Toxic to RBT & BG; not toxic to L
2,5-dichloro-4,6-dinitrophenol	Not toxic to RBT, BG, or L
3-bromo-2,4-dinitrophenol	Not toxic to RBT or L
3-bromo-4,6-dinitrophenol	ditto
Halo-trinitrophenols	
3-bromo-2,4,6-trinitrophenol	Not toxic to RBT or L
Poly-halo-mononitrophenols	
4-bromo-2-chloro-6-nitrophenol	More toxic to RBT & BrT than to L

[a] Maximum concentration tested: 5 ppm (except where otherwise noted after name of chemical)

[b] Abbreviations: RBT - rainbow trout; BrT = brook trout; BT = brown trout; BG = bluegills; L = larval lampreys

the major route of their excretion is outward diffusion via the gills. More recently, however, Lech (1974) has demonstrated that conjugation of TFM in rainbow trout and excretion in the bile is an extremely important factor in determining the fish's survival. The significance of glucuronide conjugate formation was measured by treating trout with salicylamide, a known inhibitor of glucuronide formation, before injecting the fish intraperitoneally with TFM. The results, depicted in Figure 75, clearly show that inhibiting the conjugation process increased the acute toxicity of TFM. This study indicates that, in the case of water-borne phenolic compounds, the capacity of fish for glucuronide conjugation may be an important factor in determining their survival.

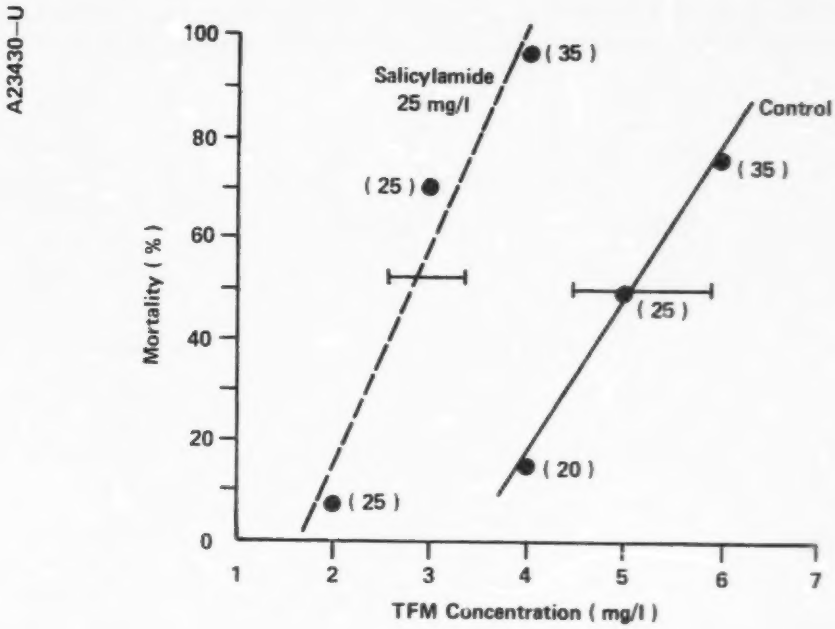


Figure 75. Enhancement of the Acute Toxicity of TFM to Rainbow Trout by Salicylamide (Lech, 1974)

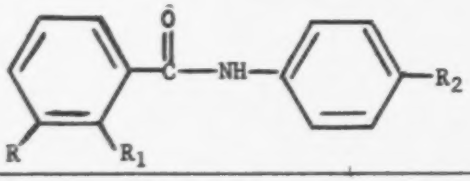
Solid line = controls; broken line = toxicity curve in presence of 25 mg/l of salicylamide. Numbers in parentheses indicate the number of animals at each point. Horizontal bars indicate 95% confidence limits.

3. Nitrosalicylanilide

In the United States today, the sea lamprey toxicant which is most commonly employed contains both TFM and 2',5-dichloro-4'-nitrosalicylanilide in a 98:2 combination (Kawatski and Bittner, 1975). The nitrosalicylanilides are known to be among the most potent uncouplers of oxidative phosphorylation ever tested (see Section III-B-4). The toxicity of these compounds to rainbow trout and sea lampreys was investigated by Starkey and Howell (1966).

The general structure-activity relationships governing selectivity and lethality by the salicylanilides are given in Tables 127, 128, and 129.

Table 127. Comparison of Molecular Requirements for Substituted Mono-halo-nitrosalicylanilides Exhibiting Selective Toxicity to Larval Sea Lamprey and Fingerling Rainbow Trout (Starkey and Howell, 1966)



Compound	Substituents			Lamprey	Trout
	R	R_1	R_2	LD_{100} (ppm)	LD_{25} (ppm)
benzanilide	..	..	..	>10.0	>10.0
4'-chlorobenzanilide	..	..	-Cl	>10.0	>10.0
salicylanilide	..	-OH	..	9.5	9.5[1]
3-nitrosalicylanilide	$-NO_2$	..	..	3.0	3.0
4'-chloro-3-nitrobenzanilide	$-NO_2$	..	-Cl	>10.0	>10.0
4'-chloro-3-nitrosalicylanilide	$-NO_2$	-OH	-Cl	0.3	0.7
4-chloro-3-acetamidosalicylanilide	$-NHCOCH_3$	-OH	-Cl	3.0	3.0[2]

---

[1] LD_{25} at 9.5 ppm

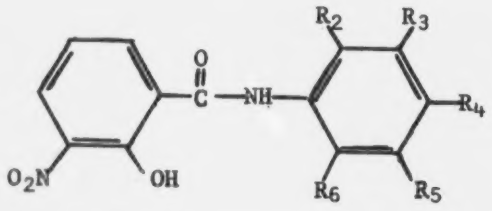
[2] LD_{100}

Table 128. Comparative Toxicity of Halonitrosalicylanilides to Larval Sea Lamprey and Fingerling Rainbow Trout as a Function of Substituent Loci (Starkey and Howell, 1966)

Compound	Lamprey LD_{100} (ppm)	Trout LD_{25} (ppm)
3'-chloro-3-nitrosalicylanilide	0.3	0.9
4'-chloro-3-nitrosalicylanilide	0.3	0.7
3'-iodo-3-nitrosalicylanilide	0.3	1.0
4'-iodo-3-nitrosalicylanilide	0.3	0.7
3'-bromo-3-nitrosalicylanilide	0.3	1.0
4'-bromo-3-nitrosalicylanilide	0.3	1.0
4'-chloro-5-nitrosalicylanilide	0.5	1.0
3'-fluoro-3-nitrosalicylanilide	0.5	0.9
4'-iodo-5-nitrosalicylanilide	0.5	1.0
4'-bromo-5-nitrosalicylanilide	0.5	1.0
2'-chloro-5-nitrosalicylanilide	0.9	3.0
2'-iodo-3-nitrosalicylanilide	1.0	3.0
2'-bromo-3-nitrosalicylanilide	1.0	1.0[1]
4'-fluoro-3-nitrosalicylanilide	1.0	3.0
2'-fluoro-3-nitrosalicylanilide	3.0	3.0
2'-chloro-3-nitrosalicylanilide	3.0	7.0
4'-fluoro-5-nitrosalicylanilide	3.0	-
3'-chloro-5-nitrosalicylanilide	15.0	15.0

[1] LD_{100}

Table 129. Selective Toxicity of Polysubstituted 3-Nitrosalicylanilides to Larval Sea Lamprey and Fingerling Rainbow Trout as a Function of Atomic Loci (Starkey and Howell, 1966)



Compound	Substituents					Lamprey	Trout
-3-nitrosalicylanilide	R_2	R_3	R_4	R_5	R_6	LD_{100} (ppm)	LD_{25} (ppm)
2' 3'-dimethyl-	$-CH_3$	$-CH_3$	..	..	..	3.0	5.0
2'-methyl-3'-chloro-	$-CH_3$	-Cl	..	..	..	0.7	1.0
2',4'-dimethyl-	$-CH_3$	..	$-CH_3$	..	..	3.0	7.0
2'-methyl-4'-chloro-	$-CH_3$	..	-Cl	..	..	0.5	0.7
2',5'-dimethyl-	$-CH_3$	..	..	$-CH_3$	..	1.0	3.0
2'-methyl-5'-chloro-	$-CH_3$	..	..	-Cl	..	0.5	0.9
2',5'-dichloro-	-Cl	..	..	-Cl	..	0.3	0.9
2'-methoxy-5'-chloro-	$-CH_3O$	..	..	-Cl	..	0.7	1.0
2',6'-dimethyl-	$-CH_3$	..	..	..	$-CH_3$	>10.0	>10.0
2'-chloro-6'-methyl-	-Cl	..	..	..	$-CH_3$	0.7	1.0

The above data indicate that an ortho-hydroxy phenolic substituent in the carboxylic acid moiety of salicylanilide is required for biological activity. In addition, maximum selectivity and lethality were obtained by the presence of a nitro group and a halogen in the same molecule.

4. Agricultural Chemicals

The problem of pollution by nitroaromatic agricultural chemicals in lakes and streams poses a serious threat to many forms of aquatic life. These chemicals, introduced into surface waters by runoff, application over water, misuse, or accident, can easily become incorporated into the aquatic food chain and ultimately affect all levels of life.

The effect of several nitroaromatic herbicides on freshwater crustaceans was studied recently by Sanders (1970). Six test species were chosen which represent important links in the food chain for fish. Bioassays were conducted under static conditions without aeration of the water. A comparison of the relative subacute toxicity of trifluralin (2,6-dinitro-N,N-di-n-propyl-α, α, α-trifluoro-p-toluidine) to crustaceans and bluegill sunfish is presented in Table 130. A large variation in the degree of toxicity to various crustaceans clearly exists for trifluralin. Nevertheless, trifluralin was one of the most toxic of the 16 herbicides tested in this study and the most toxic of all those tested against bluegill sunfish.

A time-response bioassay using scud as a test species was conducted on three nitroaromatic herbicides; dinoseb, trifluralin, and balan (N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine). The various TL_{50} values for these compounds are presented in Table 131. The decreasing TL_{50} values for both trifluralin and balan with respect to time indicate that they may act as cumulative poisons.

Table 130. Estimated 48 Hour TL_{50} Values of Trifluralin to Six Species of Freshwater Crustaceans and One Species of Fish (Sanders, 1970)

Organism and Temperature	48 hr TL_{50}[a] (mg/1)
Waterflea (Daphnia magna) 21°C	0.56
Seed shrimp (Cypridopsis vidua) 21°C	0.25
Scud (Grammarus fasciatus) 15.5°C	1.8
Sowbug (Asellus brevicaudus) 15.5°C	2.0
Glass shrimp (Palaemonetes hadiakensis) 21°C	1.2
Crayfish (Orconectes nails) 15.5°C	50.0
Bluegill (Lepomis macrochirus) 24°C	0.019[b]

[a] Median tolerance limit-concentration in water which produces a 50 percent mortality.

[b] Value reported by Cope (1966)

Table 131. Estimated TL_{50} Values and Confidence Limits (P = .05) for Several Herbicides to Scud (Sanders, 1970)

Herbicide	P,P'-DDT Reference at 48 hours (mg/1)	TL_{50} Values and Confidence Limits (mg/1)		
		24 hours	48 hours	96 hours
Dinoseb	0.0056	2.8(1.1-4.9)	2.5(1.2-4.7)	1.8(0.72-6.1)
Trifluralin	0.0032	3.2(1.9-17)	1.8(1.6-12)	1.0(0.30-3.6)
Balan	0.0022	8.2(2.4-28)	4.0(2.7-5.8)	1.1(0.61-1.9)

The herbicide dinitramine (N^3, N^3-diethyl-2,4-dinitro-6-trifluoromethyl-m-phenylenediamine) has recently been tested for toxicity to several species of freshwater fish (Olson *et al.*, 1975). The results from 96 hour static toxicity tests are presented in Table 132. The authors noted that dinitramine residues were much more persistent in fish than residues of TFM and, because of its low solubility in water, it was rapidly partitioned across the gills and absorbed into the circulation.

Table 132. Toxicity of Dinitramine (99 + %) to Nine Species of Freshwater Fish in Soft Water[a] at 12° (Olson et al., 1975)

Species	Average Weight (g)	LC_{50} and 95% Confidence Interval (mg/l) at 24 hr	LC_{50} and 95% Confidence Interval (mg/l) at 96 hr
Coho salmon (*Oncorhynchus kisutch*)	0.8	>1.51	0.600 b
Steelhead trout (*Salmo gairdneri*)	0.3	1.20 1.06-1.36	0.590 0.510-0.682
Brown trout (*Salvelinus trutta*)	0.7	1.27 1.05-1.53	0.590 0.510-0.682
Lake trout (*Salvelinus namaycush*)	0.6	1.15 0.986-1.34	0.920 0.776-1.09
Carp (*Cyprinus carprio*)	1.0	>2.00	1.18 1.02-1.36
Fathead minnow (*Pimephales promelas*)	0.8	2.63 b	1.44 1.07-1.93
Channel catfish (*Ictalurus punctatus*)	0.8	2.99 2.20-4.06	1.37 1.04-1.81
Bluegill (*Lepomis macrochirus*)	1.4	2.88 1.96-4.24	1.52 1.14-2.02
Yellow perch (*Perca flavescens*)	0.8	1.00 0.870-1.15	1.00 0.870-1.15

[a] pH 7.2-7.6; total hardness = 40-44 mg/l as $CaCO_3$

[b] Insufficient data for computation of confidence intervals

Alabaster (1969) has reported the results of fish toxicity studies using the herbicides trifluralin and dinoseb, and also the fungicide dinocap (dinitrocaprylphenol). The test results are summarized in Table 133.

Table 133. Toxicity of Trifluralin, Dinoseb, and Dinocap to Harlequin Fish (Rasbora Heteromorpha) (Alabaster, 1969)

Compound	Approximate Composition (%)	Median Lethal Concentration (ppm) 24 hr	Median Lethal Concentration (ppm) 48 hr	Estimated Threshold (ppm)	Water Type
Dinoseb	9.0	3.4	3.0	2.7	tap
Trifluralin	46	1.0	0.6	0.35	soft
		0.6	-	-	tap
Dinocap	25	0.39	0.27	0.13	soft

Fabacher and Chambers (1974) have recently demonstrated that resistance to herbicides is possible in insecticide-resistant fish. Drainage canal mosquito fish exposed to dinoseb or trifluralin displayed LC_{50} values of 0.96 and 4.10 ppm, respectively, whereas farm pond mosquito fish had LC_{50} values of 0.87 and 2.00 for the same compounds. These results showed a 2.05 fold resistance to trifluralin in the drainage canal fish and is a unique example of fish resistance to herbicides.

Approximate toxicity ranges for DNOC, dinoseb, and dinocap to freshwater fish have been established by the British government (Mawdesley-Thomas, 1971). The range within which aqueous concentrations are lethal to fish were given as above 1.0 to 10 ppm for DNOC, above 0.1 to 1.0 ppm for dinoseb, and above 0.01 to 0.1 ppm for dinocap. These tests, however, were of short duration (24 and 48 hours) and do not provide very reliable data.

It has been suggested by many investigators that aquatic bioassays be conducted over a minimum period of 96 hours.

The fungicide 2,6-dichloro-4-nitroaniline (DCNA) was evaluated for toxicity using both fish and wildlife (Knott and Scott, 1968). The LC_{50} (50% mortality) values for fish were derived from 48 hour and 96 hour bioassays, while mortality in bobwhite quail and mallard ducks was determined by subacute toxicity studies. Their results are presented in Table 134.

Table 134. Mortality in Fish and Wildlife by DCNA, DDT, and Diphenamid (Knott and Scott, 1968)

Species	LC_{50} (ppm active ingredient)			
	Diphenamid	p,p'-DDT	DCNA	p,p'-DDT
Bobwhite quail	>9,000	486	2,438	486
Mallard ducks	15,000	525	8,850	525
Rainbow trout	8.6	0.0028	1.6	0.0032
Bluegill sunfish[a]	>32.0	0.0022	37.0	0.0020
Goldfish[a]	34.0	0.0025	>32.0	0.0049

[a] 96 hour mortality

5. Trinitrotoluene

A comprehensive review of the toxicity of munitions-related compounds was made by Dacre and Rosenblatt (1974) and includes toxicity of the nitrotoluene derivatives to aquatic organisms. In this review, they cite toxicity data for TNT and related compounds to 5-8 cm "minnows" at 23-24°C (Table 135).

Table 135. Acute Toxicity of Several Nitroaromatic Compounds to Fish (Dacre and Rosenblatt, 1974)

Compound	6 Hour Minimum Lethal Dose (mg/l)	
	Hardness, 12.5 ppm $CaCO_3$	Hardness, 150 ppm $CaCO_3$
Trinitrotoluene	4.0-5.0	4.0-5.0
m-Dinitrophenol	0.5-1.0	35-38
Dinitro-o-cresol	1.5-2.0	3.0-4.0
o-Mononitrotoluene	18-20	35-40
m-Mononitrotoluene	14-18	25-30
p-Mononitrotoluene	20-22	45-50

These comparative data show that TNT has a greater acute toxicity (measured as mortality) than the mononitrotoluenes but still is considerably less toxic than dinitrophenol. These findings are consistent with toxicity data obtained from mammalian studies, which indicated that increasing the number of nitro groups raised the toxicity of nitrobenzene derivatives, but that these non-phenolic chemicals were still less toxic than dinitrophenols (see Section III-D-1).

These authors also noted that dinitrotoluene (isomer unknown) had 24, 48, and 96 hour median tolerance levels in bluegills of 50, 27, and 16 mg/l respectively.

Nay (1974) has recently shown that bluegills exposed to TNT displayed a median tolerance limit of 2.6 mg/l in 96 hour static bioassay tests, indicating a stream standard of 0.02 mg/l.

An extensive evaluation of the toxicity of TNT wastes on bluegills was conducted by Pedersen (1970). Calculations were made of the LC_{50} (median lethal concentration) of alpha-TNT for bluegills in specific time periods and under varying conditions of water hardness and temperature. These results are summarized in Figure 76.

Figure 77 depicts the percent mortality after 96 hours versus TNT concentration in four different bioassays. Statistical analysis revealed that water hardness did not alter toxicity but that temperature did.

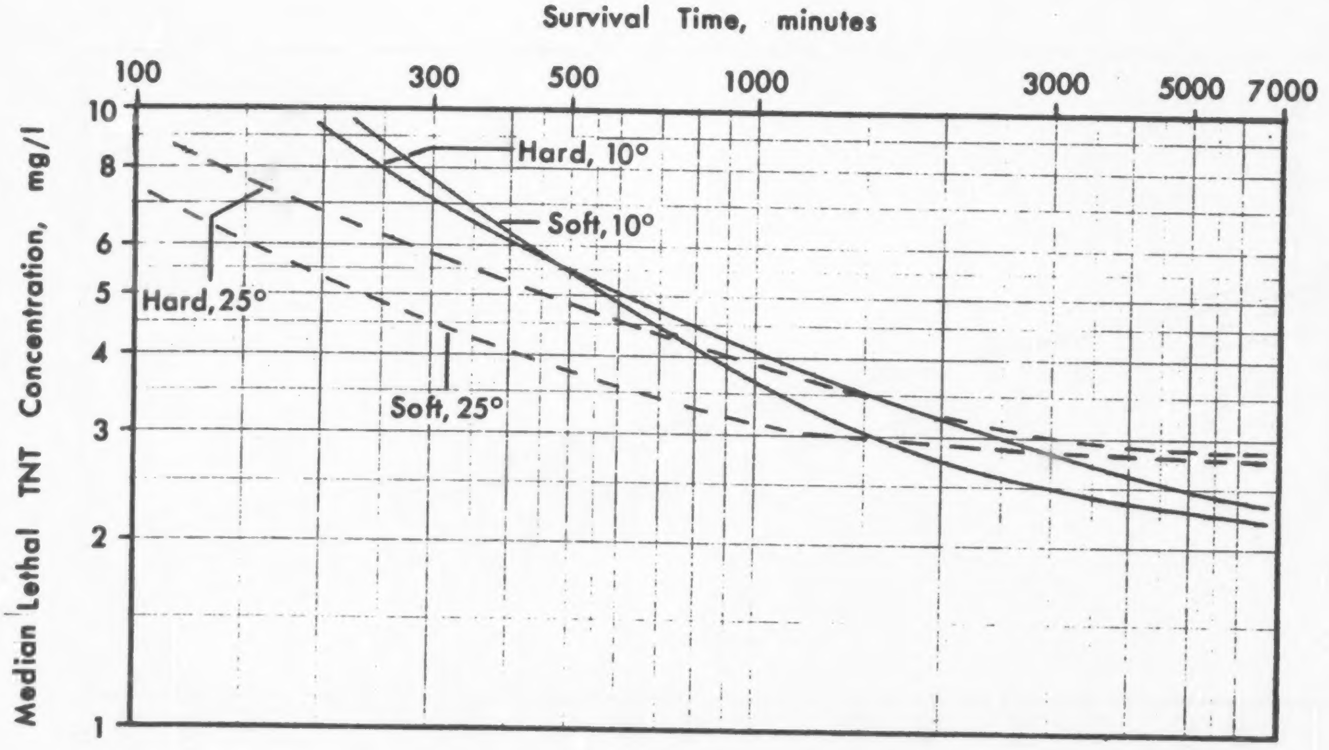


Figure 76. Relative Susceptibility of Bluegills to TNT at Different Water Hardness* and Temperature (Pederson, 1970)

*Hard water, 180 ppm as $CaCO_3$; soft water, 60 ppm as $CaCO_3$.

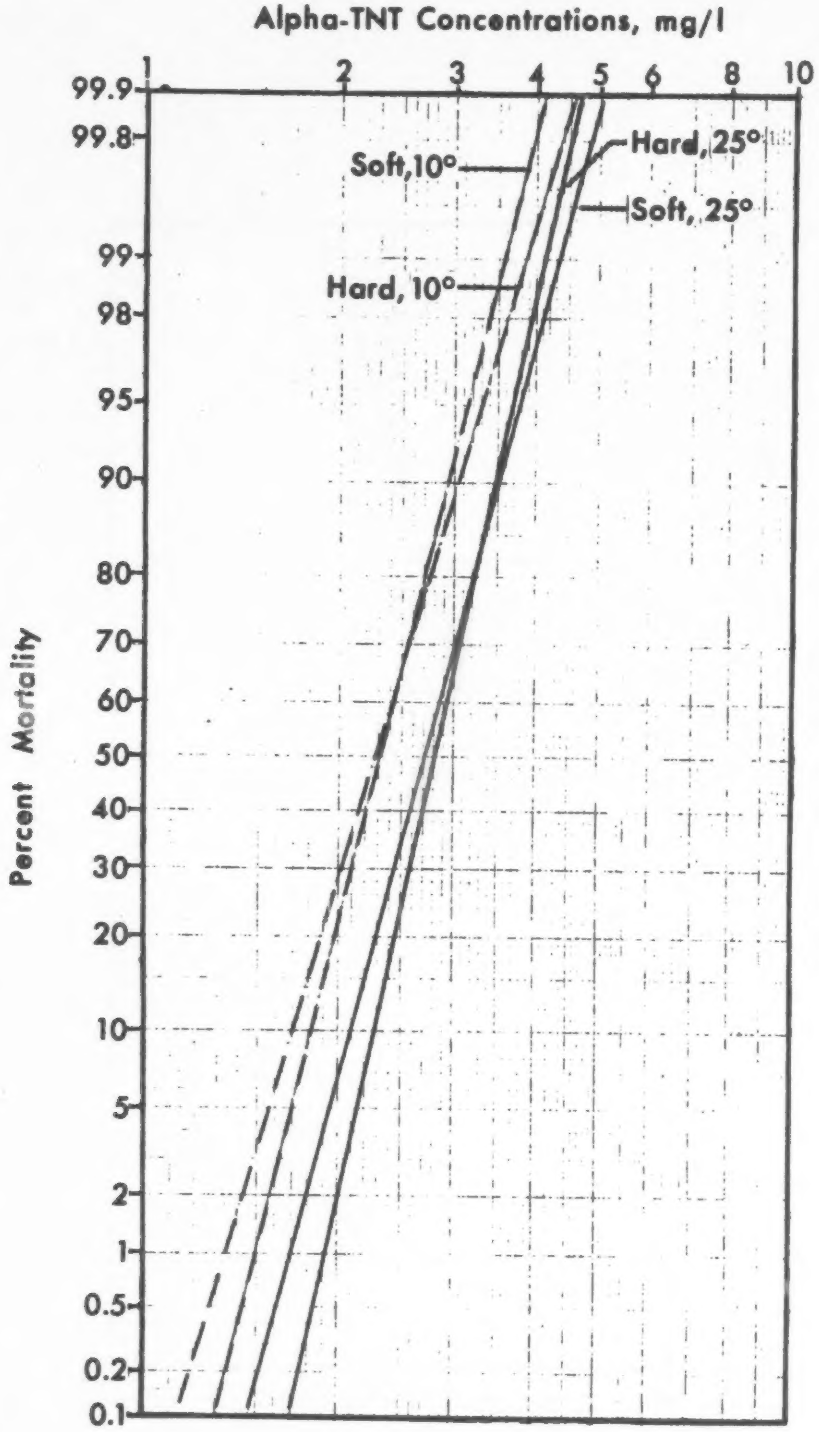


Figure 77. Mortality Curves at Four Days (Pederson, 1970)

F. Toxicity - Plants

Only limited amounts of information are available concerning the toxicity of nitroaromatic compounds to higher plants. The chemical groups which have received some attention are nitrophenols, nitrobenzenes, and nitrotoluenes. Again, nitrophenols have been most extensively investigated perhaps because of the interest of researchers in these compounds for their ability to specifically uncouple oxidative phosphorylation from electron transfer. Furthermore, a number of nitrophenolic compounds are used as pest control agents and understanding the mechanism of their action has been important from the point of view of developing new and specific pest control agents.

1. Nitrophenols

An extensive study of the effect of *o*- and *p*- nitro, 2,4-dinitro and, 2,4,6-trinitrophenol has been carried out by Simon and Blackman (1953). Phytoxicity was studied using three different assay systems: (1) Inhibition of the growth of the duck weed, *Lemna minor*, (2) Inhibition of the respiration of vacuum infiltrated leaf disc of *Brassica alba*, and (3) Mortality of seedlings of *Brassica alba*. The concentration of each substance required to halve the growth rate, respiration, and mortality, respectively, were determined. Since the degree of ionization affects the toxicity of nitrophenols, in the measurements above, pH values below pKa (where there is little ionization) were used. The results of this study are presented in Table 136. As can be seen, nitrophenols at low concentrations inhibited growth of *Lemna minor*, and respiration of the leaf discs of *Brassica alba*. The seedlings of *Brassica alba* were affected only at high concentrations of the toxicants; however, the results are inconclusive since nitrophenols were sprayed on the seedlings and there may well

Table 136. Relative Toxicity of Mono- and Dinitro-substituted Phenols on Plants (Simon and Blackman, 1953)

Compound tested	Concentrations Required For		
	50% reduction of growth rate of *Lemna minor* M	50% reduction of respiration rate of infiltrated leaf disks of *Brassica alba* M	50% mortality of seedlings of *Brassica alba* M
o-nitrophenol (pK = 7.3)	4.5×10^{-4} (5.4)**	1.6×10^{-3} (3.0) 1.2×10^{-3} (3.0)	> 2.2×10^{-1} (3.0) > 3.6×10^{-1} (3.0)
p-nitrophenol (pK = 7.2)	6.8×10^{-5} (5.2)	2.5×10^{-4} (3.0)	1.2×10^{-2} (3.0) > 9×10^{-3} (3.0)
2,4-dinitrophenol (pK = 4.0)	8.0×10^{-6} (5.2)	1.3×10^{-5} (3.0)	1.5×10^{-2} (3.0)
2,4,6-trinitrophenol (pK = 0.8)	2.4×10^{-4}* (5.1) 7.6×10^{-3}* (5.1)	-	-

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* Results with two batches of picric acid.

** The pH at which toxicity was measured is given in parentheses after each figure.

have been significant losses of the chemicals. Of interest was the observation that the relative toxicity of nitrophenols in all three test systems was similar. For example, p-nitrophenol was always more toxic than the o-isomer. The utility of the toxicity data in terms of the environmental effects is hard to assess because of the unusually low pH values used in the studies. At lower pH, it is questionable if the physiological state of the cell is normal and consequently if response to the toxicant is normal. Since the toxicity of nitrophenols decreases as the pH is raised above the pK, at physiological pH values the toxicity will perhaps be much lower than that determined by Simon and Blackman (1953). Their results could at best be indicative of the upper limit of the toxicity of nitrophenols.

Seedling emergence in Vicia faba was effectively inhibited by p-nitrophenol (0.1%) but not by the o-isomer (Amer and Ali, 1968). Both the isomers, however, were able to cause appreciable injury when sprayed on the plants. Among the other seeds tested, p-nitrophenol was found to inhibit germination of Tritricum vulgare, Gossypium barbadense, Pisum sativum, and o-nitrophenol inhibited T. vulgare and G. barbadense. Similar to the observations of Simon and Blackman (1953), these results also suggest that the p-isomer is relatively more toxic than the o-isomer.

A number of researchers have studied the effect of DNP on uptake and respiration of exogenously supplied substrates in plants. In sunflower hypocotyls, Reinhold and Eilam (1964) measured the exogenous respiration by supplying the hypocotyls with $^{14}CO_2$ evolved. 2,4-Dinitrophenol (DNP) was reported to drastically reduce the exogenous respiration while the overall CO_2 production (CO_2 evolved from endogenous + exogenous substrate) was actually increased (Table 137). DNP (1.4×10^{-4} M) also raised the Respiratory Quotient for the tissue from 1.0 to 1.2. Since the specific activity of the $^{14}CO_2$ evolved decreased in the presence of DNP, it was suggested that DNP reduced the contribution of exogenous ^{14}C-labelled substrate in the CO_2 given off. Humphreys and Dugger (1959), working with corn roots, noted that DNP inhibited the uptake of exogenous substrates. Such an effect could be attributed to the disorganization of the cell membranes by DNP (Reinhold and Eilam, 1964).

Table 137. Effect of 2,4-Dinitrophenol (pH 5.8) on Exogenous Respiration by Segments of Sunflower Hypocotyl (Reinhold and Eilam, 1964)

Exogenous Substrate	DNP Concen. (M)	Percent Change Over Control		
		Overall CO_2 evolved	$^{14}CO_2$ evolved	Specific activity of $^{14}CO_2$*
^{14}C-Glutamic acid 2×10^{-3} M	1.6×10^{-4}	+37	-27	-47
	3.2×10^{-4}	+19	-31	
^{14}C-Glucose 5×10^{-3} M	2×10^{-4}	+71	-44	-67

*Specific activity indicates the amount of exogenous substrate respired relative to the total substrate.

From the studies described above, it is clear that nitrophenols are toxic to plants. They interfere with the uptake and respiration of substrates, inhibit plant growth, and cause mortality in seedlings at higher concentrations. Similar to the observations with other organisms, the toxicity in plants increased in the series mononitrophenol, dinitrophenol, but decreases with a third nitro group. Among mononitro-phenols, the p-isomers are generally more toxic than the o-isomers in plants.

2. Nitrotoluenes

The phytotoxicity of some products of TNT manufacture (mono-, di-, and trinitrotoluenes, and one transformation product, 4-amino-2-nitrotoluene) has been investigated by Schott and Worthley, 1974 (Table 138). In the aquatic flowering plant *Lemna perpusilla*, both 2,4-dinitro- and 2,4,6-trinitrotoluene depressed growth or killed colonies at concentrations of 1 ppm or above; 4-amino-2-nitrotoluene was inhibitory to the test organism only at concentrations of 10 ppm or higher. On the other hand, o-nitrotoluene showed no harmful effect in the test organisms except when the concentration reached 100 ppm. The response of the organism to the nitrotoluenes in acidic (6.3 pH) and basic medium (8.3 pH) was similar except in the case of 4-amino-2-nitrotoluene which was slightly more toxic in alkaline than in acidic medium. Upon comparison of the response of *Lemna perpusilla* to a known plant toxicant, 2,4-dichlorophenoxyacetic acid, it was noted that dinitro- and trinitro-substituted toluenes were approximately one tenth as toxic as the herbicide.

A field study to evaluate the effect of discharges from Radford Army TNT plant (located on the New River, Virginia) on submerged and emersed aquatic vegetation has been carried out by Mitchell (1973). The author surveyed the number of species of aquatic flowering plants, mosses, and "stemmed" algae of the phylum *Charophyta*, and determined the relative biomass upstream (designated as reference) and downstream from the plant discharge. A change in plant population (species distribution or in total biomass) was attributed to the introduction of TNT waste. Ten stations were chosen at the river bank to

Table 138. Summary of Lemna perpusilla Colony Growth When Exposed to Various Nitrotoluenes (Schott and Worthley, 1974)

Compound	Test pH	Plant responses*								
		Concentrations (ppm)								
		100	50	10	5	1	0.5	0.1	0.01	0.001
o-Nitrotoluene	6.3	D	–	0	–	0	–	0	–	–
o-Nitrotoluene	8.5	0	0	0	–	0	–	–	–	–
4-Amino-2-nitrotoluene	6.3	D	X	0	–	0	–	–	–	–
4-Amino-2-nitrotoluene	8.5	X	X	X	–	0	–	–	–	–
2,4-Dinitrotoluene	6.3	D	–	D	–	X	X	0	0	–
2,4-Dinitrotoluene	8.5	D	D	D	D	X	–	0	–	–
2,4,6-Trinitrotoluene	6.3	–	D	D	D	X	0	0	0	–
2,4,6-Trinitrotoluene	8.5	–	D	D	D	X	–	0	–	–
2,4-Dichlorophenoxy-acetic acid (Positive Control)	6.3	–	–	D	–	D	–	X	0	0

*D = Death; X = Decrease in growth rate; 0 = no effect; – = not tested.

evaluate the individual effects of various discharges; only stations 5P and higher numbers were located downstream from the TNT manufacturing site. Interpretation of the results to determine the effect of TNT waste alone is complicated because these stations were also receiving effluents from an oleum plant and a power plant located upstream from the TNT plant (see Figure 78). The changes noted at station 5P may actually be collective rather than due to the TNT waste alone. At best, rough conclusions concerning the harmful effect of TNT waste can be drawn considering station 4P as the reference. If a species is present at station 4P but cannot be detected at stations 5P or beyond, it

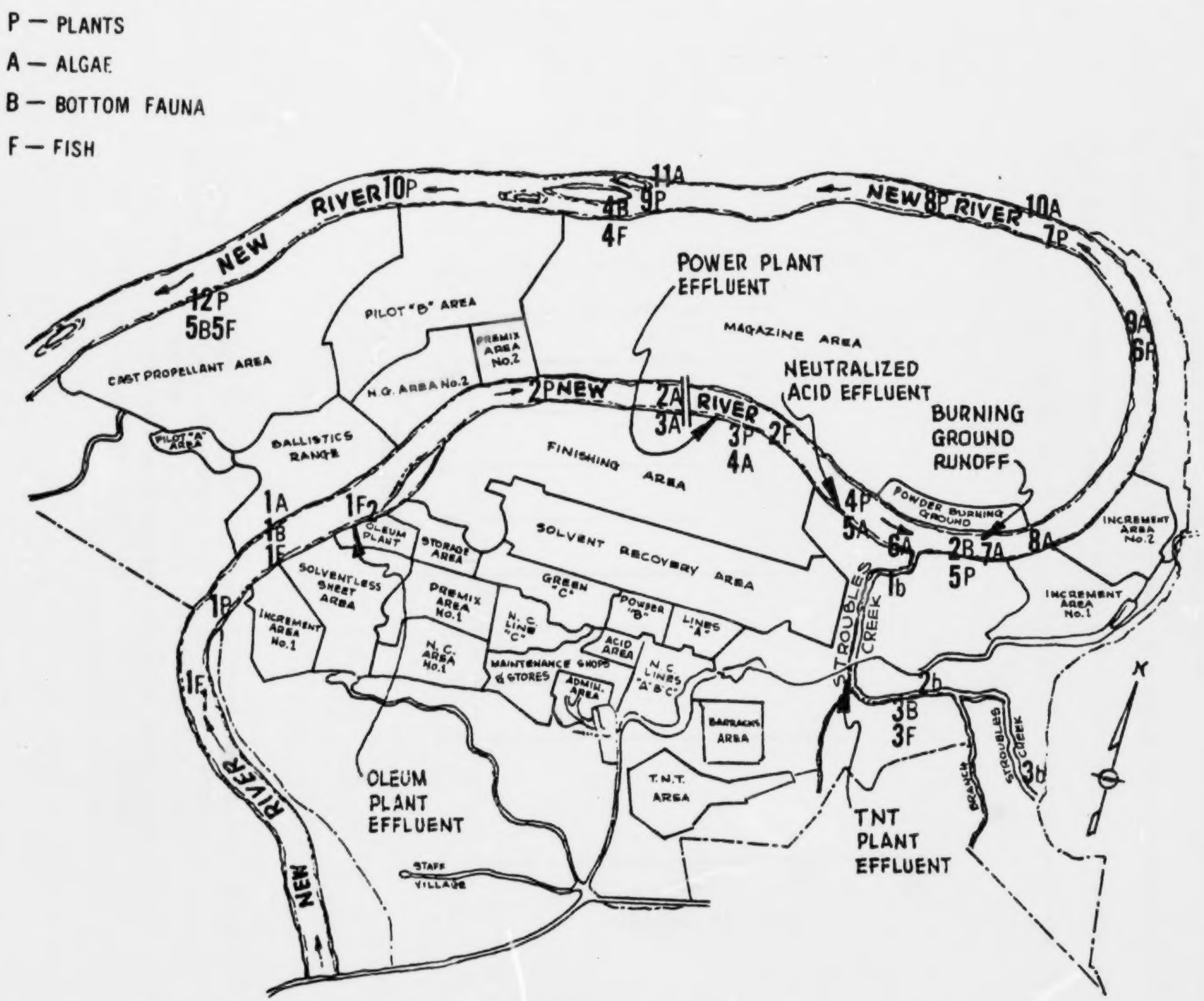

Figure 78. The Effect of Waste Discharges from Radford Army Ammunition Plant on the Biota of the New River, Virginia - Location of Sampling Points (Mitchell, 1973) (Reprinted with permission from the Water Resources Research Center, Virginia Polytechnic Institute and State University.)

could safely be attributed to the introduction of TNT waste. The results indicated a decrease in the number of species by about one third at the point of TNT plant effluent (Figure 79). The species which were characteristically absent in the area of waste discharge are shown in Table 139.

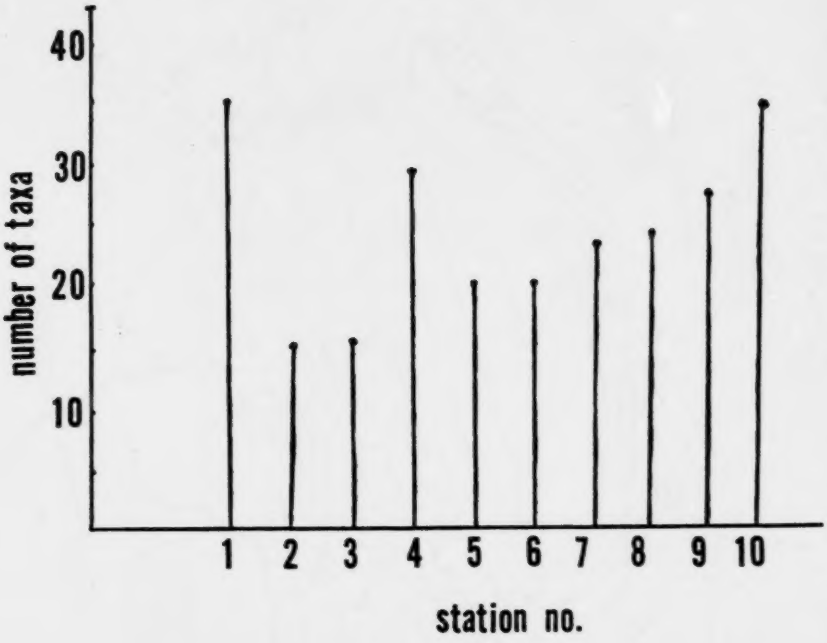

Figure 79. Histogram Representing the Number of Species of Aquatic Plants Present Below the High-Water Mark in Ten Samples Along the New River, June 1971 (Mitchell, 1973) (Reprinted with permission from the Water Resources Research Center, Virginia Polytechnic Institute and State University.)

Table 139. Plant Genera and Species Affected by the Introduction of TNT Plant Effluent in New River (Virginia) (Radford Army Ammunition Plant) June, 1971 (Mitchell, 1973)

Plant Genera or Species	Station*						
	4P Upstream from TNT plant (Reference)	5P	6P	7P	8P	9P	10P
		Downstream from TNT plant					
Compositae							
Bidens frondosa L.	+	-	-	-	+	-	+
Rudbeckia laciniata L.	+	-	-	-	-	-	+
Cornaceae							
Cornus amomum L.	+	+	-	-	+	-	-
Cruciferae							
Arabis laevigata (Muhl.) Poir.	+	-	-	-	-	-	-
Cyperaceae							
Eleocharis obtusa (Willd.) Schultes	+	-	-	-	-	+	+
Gramineae							
Echinochloa crysgalli (L.) Beauv.	+	+	-	-	-	-	+
Panicum sp. (not flowering)	+	+	-	-	-	-	-
Hypericaceae							
Hypericum mutilum L.	+	-	-	-	-	-	-
Labiatae							
Mentha arvenis L.	+	-	-	-	-	-	-
Onagraceae							
Ludwigia palustris (L.) Ell.	+	-	-	-	-	-	+
Rosaceae							
Agrimonia gryposepala Wallr.	+	-	-	-	-	-	-
Solanaceae							
Solanum carolinense L.	+	-	-	-	+	+	+
Urticaceae							
Laportea canadensis (L.) Wedd.	+	-	-	-	-	+	-

Key:

- indicates absence of taxa
+ indicates presence of taxa
* see map, Figure 78

As the river left the discharge site, a number of these species reappeared, and there was a gradual increase in the total number of taxa. By the time the river approached sampling site 10P the number of species had returned to more or less that of the reference. In this study, the authors also determined the relative biomass at the sampling stations. However, the data is of little use to assess the toxicity of TNT waste because the biomass, by the time the river reached station 5, had already been severely affected by discharge from other effluents upstream from the TNT waste discharge site.

In view of the fact that (1) nitro-substituted toluenes are toxic to duckweed, an important member of the food web and that (2) the waste from the TNT plant causes considerable changes in the species distribution in aquatic vegetation, it can be said with certainty that nitro-substituted toluenes will have an adverse effect on the aquatic and surrounding environment.

3. Nitrobenzenes

The toxicity of nitro-substituted benzenes to higher plants has received very little attention. However, the effect of m-dinitrobenzene on photosynthesis in Chlorella and spinach chloroplast has been examined. Among the two phases of fluorescence induction (photochemical and thermal), the thermal phase was reported to be inhibited at concentrations lower than 10^{-4} M. The authors attributed the inhibition to an irreversible binding of the toxicant to the reaction centers. At higher dinitrobenzene concentrations, the photochemical phase appeared to have been inhibited. The data suggest that m-dinitrobenzene may adversely affect plants by interfering with their photosynthetic process.

G. Toxicity - Microorganisms

A considerable amount of work has been done to determine the toxicity and mechanisms of action of nitro-substituted aromatic compounds. Unfortunately, in almost all of the reported studies, the concentrations of the toxicants used are much higher than, and therefore do not simulate, field concentrations. One of the main reasons for this appears to be the limited sensitivity of the parameter chosen to study the response of the organism. The types of microorganisms which have been used in studying the toxicity of nitroaromatics include yeast, molds, and unicellular algae. Some studies have dealt with the effect of nitroaromatic compounds on the activity and performance of activated sludge. Nitrophenolic compounds (particularly 2,4-dinitrophenol) have been studied much more extensively than any other group of nitroaromatics, perhaps because of the interest of researchers in these compounds due to their specific uncoupling effect. In this section, effort has been made to summarize all the literature concerning the effect of nitroaromatics on microorganisms. The available information is organized by the type of organisms which have been used in the toxicity studies, e.g., bacteria, yeast, unicellular algae, etc.

1. Effects on Bacteria

Studies dealing with the effect of nitroaromatic compounds on bacteria are summarized in Table 140. As can be noted, a majority of the studies falling in this section deal with the effect of nitrophenols, particularly 2,4-dinitrophenol, a long-known uncoupler of oxidative phosphorylation. The compound effectively disconnects the oxidative phosphorylation energy transferring sequence from the electron transferring sequence, and thus deprives the cell of the available energy. The susceptibility of bacteria to the toxic action

Table 140. Summary of the Studies Dealing with Toxicity of Nitroaromatics to Bacteria

Reference	Test Chemical Studied	Concentration Employed	Test Organism	Cell Function Studied	Analytical Method
Allwood and Hugo, 1971	2,4-Dinitrophenol	0.1 mM	*Staphylococcus aureus*	1. Leakage of cations and amino acids 2. Cell inactivation	Cations by flame photometer, amino acids by ninhydrin method; cell inactivation by pour plate method
Kerridge, 1960	"	1 mM	*Salmonella typhimurium*	1. Regeneration of flagella 2. Protein and nucleic acid synthesis	Motility estimation and flagellar staining; total nucleic acid from extinction at 260 mμ; DNA by diphenylamine method; protein colorimetrically with Folin reagent
Aboud and Burger, 1972	"	0.125 mM	*Escherichia coli*-K12	β-Galactosidase synthesis	Assay of the enzyme, total protein synthesis by measuring ^{14}C-amino acid incorporation
Bauerle and Bennett, 1960	"	25-250 ppm	*Pseudomonas aeruginosa* isolated from spoiled emulsion oil	Oxidation of C_4, C_6, C_8, C_{10}, C_{12}, C_{13}, C_{14}, C_{16} saturated aliphatic fatty acids; cell growth	Oxygen uptake by Warburg manometric technique, growth by measuring cell numbers
Rieder and Bukatsch, 1956	2,4-Dinitrophenol	10^{-3} - 10^{-6} M	Luminous bacteria: *Photobacterium phosphoreum*	Glowing of bacteria	Measure light intensity
Cowles and Klotz, 1948	Several mono-, di-, and trinitro substituted phenols; amino substituted nitrophenols	1×10^{-3} - 14×10^{-3} M	*Escherichia coli*, *Bacillus mesentericus*	Cell multiplication	Visible growth

Table 140. Summary of the Studies Dealing with Toxicity of Nitroaromatics to Bacteria (Cont'd)

Reference	Test Chemical Studied	Concentration Employed	Test Organism	Cell Function Studied	Analytical Method
Cooper and Mason, 1927, 1928	2,4,6-Trinitrophenol (picric acid)	200 - 3500 mg/liter	*E. coli* *P. fluorescens* *S. marcescens* *B. mesentericus* etc.	Growth	-
Demerec *et al*., 1951	2,4,6-Trinitrophenol	100 - 180 mg/liter	*E. coli*	Mutagenic action	-
Mohn, 1971	Pentachloronitrobenzene	2 mg/ml	*E. coli* 343, (a non-inducible, galactose negative mutant)	Forward mutation to galactose prototrophy	Spot test on galactose minimal medium
Clarke, 1971	Pentachloronitrobenzene	10 - 15 mg/bacterial seeded plate	*E. coli* B/r ochre auxotropic mutant WWP-2	Reversion to tryptophan independence	Scoring the colonies of revertants

of nitroaromatics has been ascertained by studying the effect on cell growth and multiplication, and the effects on sensitive cell functions, such as permeability, enzyme synthesis, oxidative ability, etc. For certain chemicals, the mutagenic potential in bacteria has also been evaluated.

a. Growth Inhibition

One of the earlier studies dealing with the bactericidal activity of certain nitrophenols is that of Cooper and Mason (1927, 1928). They studied the influence of picric acid on a number of bacteria, including _E_. _coli_, _Pseudomonas_ _fluorescens_ and many others. Although picric acid was inhibitory to all the bacteria tested, the effective concentration differed from one organism to another (ranging from 125 mg/ℓ to 3330 mg/ℓ, depending upon the organism and the period of exposure).

Cowles and Klotz (1948) have investigated the toxic action of 2,4-dinitrophenol in microorganisms in some detail. In this study, cultures of _Escherichia_ _coli_ and _Bacillus_ _mesentericus_ were used as the test organisms, and for media both complex broth solution and simple chemically defined media were used. The bacteriostatic value was taken as the lowest concentration of the test chemical which prevented visible growth for 4 days. The bacteriostatic values with the two bacteria on either medium were found to be essentially similar, and therefore, only the data obtained with _E_. _coli_ grown in yeast extract broth are given in Table 141. The bacteriostatic concentrations of the compounds tested were found to be in the millimolar range, which is far above reasonable environmental concentrations. It is unlikely, therefore, that at environmentally significant concentrations the nitroaromatic compounds will have any bacteriostatic effect. The data further reveals that the toxicity of

Table 141. Bacteriostatic Activity of Certain Nitrophenols (Cowles and Klotz, 1948)

Test Compound	pKa Constant	Bacteriostatic Concentration* (Molar x 10^{-3})
m-nitrophenol	8.3	2.8
p-nitrophenol	7.1	2.5
2-amino,4-nitrophenol	7.0	8.0
2,5-dinitrophenol	5.1	1.0
2-amino,4,6,-dinitrophenol	4.4	11.0
2,4-dinitrophenol	4.0	5.6
2,6-dinitrophenol	3.6	11.0
2,4,6-trinitrophenol	0.8	14.0

* Lowest concentration which prevented growth for 4 days. The data given are for culture pH 7.5.

nitrophenols, in the concentration range examined, decreases with an increase in the number of nitro-groups on the molecule. In other words, the order of toxicity is mono->di->tri-, except that the 2,5-substituted dinitrophenol was a stronger inhibitor than any of the mono-nitro substituted phenols tested. When the bacteriostatic action of various nitrophenols was examined in culture medium at various pH conditions (5.5 - 8.5), it was shown that the effectiveness of nitrophenols increased with a fall in pH. These observations led the authors to conclude nitrophenols owe their activity to the undissociated form.

Fujita (1966) applied a structure-activity correlation to the data of Cowles and Klotz (1948) in order to assess the physiological activity of nitrosubstituted phenols which exist partly as neutral molecule and partly in the ionized form at physiological pH. The bacteriostatic activity was correlated with the chemical structure using the Hammet σ constant and a substituent constant π for lipohydrophilic character of the molecule. The analysis of the data revealed that perhaps both the electronic as well as the lipohydrophilic character of the substituent were important in determining the toxicity of nitrophenolic compounds. More definite conclusions could not be drawn because the data for a wide variety of phenols were not available.

The influence of o-nitrobenzoic acid on reproduction and growth of microorganisms has been studied by Durham and his coworkers. Montgomery and Durham (1970) reported strong inhibition (about 94%) of the growth of Pseudomonas fluorescens by o-nitrobenzoic acid (120 mM). The results were unchanged whether the growth medium contained succinate or protocatechuate, as carbon source, in the latter case the induced synthesis of protocatechuate oxygenase being a requisite. Kirkland and Durham (1963) noted the inhibition of growth by o-nitrobenzoic acid of Flavobacterium using p-nitrobenzoic acid as the sole source of carbon. In the above studies, the concentration of the toxicant used was very high. Although growth inhibition was observed at these concentrations, it is debatable if a significant degree of growth inhibition will occur at low environmentally conceivable concentrations.

b. Effect on Cell Permeability

Allwood and Hugo (1971) reported an adverse effect of 2,4-dinitrophenol (2,4-DNP) on the permeability of the bacterium, Staphylococcus aureus. They found that addition of concentrations of dinitrophenol as low as 0.1 mM to the suspension of cells caused a marked increase on K^+ loss from the cells. The efflux increased with an increase in concentration of the toxicant. The minimum concentration of 2,4-DNP which was effective in causing loss of

K^+ also resulted in 20% loss of viability. No amino acid leakage was observed after treatment with dinitrophenol under similar conditions. The loss of Na^+ was also unaffected by the presence of dinitrophenol. However, Na^+ loss was very rapid and difficult to measure accurately, and therefore, these results are inconclusive.

c. Effect on Protein Synthesis

Since flagella from Salmonella typhimurium have been shown to consist of a single well-characterized protein (Kauffmann, 1954; Ambler and Rees, 1959), regenerating flagella have provided a suitable system to many researchers for studying the effect of toxicants on protein synthesis. Kerridge (1960) used this system to investigate the effect of 2,4-dinitrophenol. A culture of Salmonella typhimurium was made non-flagellate by mechanical removal of their flagella, and used in the regeneration studies. Although 2,4-DNP (10^{-3} mM) had no effect on the motility of the normal flagellated bacterium, the toxicant completely inhibited regeneration of flagella by mechanically deflagellated S. typhimurium (Table 142). Direct measurement of the cellular protein and nucleic acid levels in the treated cell further confirmed that 2,4-DNP was an effective inhibitor of protein synthesis

The inhibitory effect of o-nitrobenzoic acid on microbial protein synthesis has been noted by Montgomery and Durham (1970). They found that the synthesis of inducible enzymes, protocatechuate oxygenase in Pseudomonas fluorescens, and of β-galactosidase in E. coli, was delayed by o-nitrobenzoic acid. The inhibitor at a concentration of 13 mM caused nearly 68% and 78% inhibition of protocatechuate oxygenase and β-galactosidase, respectively. At this concentration of o-nitrobenzoic acid, no effect on cell viability or on the

Table 142. Effect of 2,4-Dinitrophenol on the Regeneration of Flagella, and on Protein and Nucleic Acid Synthesis by Salmonella typhimurium (Kerridge, 1960)

Concn. of 2,4-DNP tested (M)	Regeneration of Flagella		% Inhibition of Synthesis		
	Motile Bacteria (%)	Flagella/ Bacterium (mean)	Total Nucleic Acid	DNA	Protein
-	90	3.6	-	-	-
5×10^{-4}	50	2.1	80	70	54
1×10^{-3}	1	-	100	100	94
2×10^{-3}	1	-	100	100	100

activity of the existing enzyme was observed. At 10 fold lower concentrations of o-nitrobenzoic acid, the synthesis of protocatechuate oxygenase was unaffected; the effect of the lower toxicant concentration on β-galactosidase synthesis was not studied. The sensitivity of the inducible enzymes to o-nitrobenzoic acid suggests that the compound interferes with the synthesis of protein. This conclusion gains support from the finding that o-nitrobenzoic acid also inhibits the incorporation of radioactive amino acids into protein (TCA insoluble fraction) (Montgomery and Durham, 1970).

The inhibition of protein synthesis (i.e., flagella, inducible enzymes, etc.) by toxic chemicals could have many environmental consequences. For instance, the non-flagellated cells will fail to respond to the stimulus provided by a chemical substance (chemotactic response) which is introduced in the environment. A chemotactic response is generally regarded as a prerequisite for microbial attack on a chemical. Therefore, it may be reasonable to conclude that the presence of an inhibitor of flagella synthesis will affect the biodegradability of chemical substances in the environment. Alexander (1973) has stated that enzymes participating in the initial phases of decomposition of a number of synthetic and natural products require induction. From this, it appears that toxicants which interfere with protein synthesis (i.e., formation of inducible enzymes) will also have an impact on biodegradability of chemical substances. However, from the data available, it cannot be said if 2,4-dinitrophenol or o-nitrobenzoic acid will have such an effect under field conditions and at low environmentally significant concentrations.

d. Influence on Oxidative Enzyme Systems

An extensive investigation concerning the effect of 2,4-dinitrophenol on the oxidation of saturated aliphatic fatty acids (C_4 - C_{18}) by *Pseudomonas sp*. was carried out by Bauerle and Bennett (1960). A pure culture of *Pseudomonas aeruginosa* isolated from a spoiled emulsion oil was the test organism. The results of the manometric studies indicated that the oxygen uptake with nearly all fatty acids increased slightly in the presence of 2,4-dinitrophenol (Table 143). However, similar concentrations of 2,4-DNP resulted in inhibition of growth (growth studies were done only with capric acid). The authors concluded that even though DNP stimulates oxygen uptake, the compound has an inhibitory effect upon multiplication of the organism. It should be added here that most compounds which act by uncoupling oxidative phosphorylation from respiration are known to act in this manner.

Table 143. Effect of 2,4-Dinitrophenol on the Oxidation of Saturated Aliphatic Fatty Acids by Pseudomonas aeruginosa (Adapted from Bauerle and Bennett, 1960)

Substrate	% Inhibition(-) or Stimulation(+) in 6 Hours at DNP Concentrations	
	25ppm	100ppm
None (endogenous)	+12	+14
Butyric acid	+ 9	- 3
Caproic acid	+ 9	+14
Caprylic acid	+ 3	+ 8
Capric acid	+ 1	+11
Lauric acid	0	+ 4
Tridecanoic acid	+15	+21
Myristic acid	0	+ 5
Palmitic acid	+ 4	- 2

e. Mutagenic Effects

So far, only a few nitroaromatic compounds have been tested for mutagenic potential. The criterion used by Clarke (1971) in determining mutagenicity of pentachloronitrobenzene (PCNB) was reversion of tryptophan independence in the E. coli B/r ochre auxotrophic mutant WWP-2. In this test, PCNB was found to be detectably mutagenic in the hcr^- (excision repair deficient) derivative of the strain. In the hcr^+ strain (excision repair competent), the compound was not measurably mutagenic. PCNB, however, could not be shown to be mutagenic in another strain of E. coli with a different system (Mohn, 1971) (i.e., forward mutation to galactose prototrophy in a phenotypic galactose negative strain of E. coli). The concentration of PCNB was 2 mg/ml in this test. Clarke (1971) used 10-15 mg PCNB/plate in his test. Since exact toxicant concentration in the latter case is not known, comparison of the results from the two sources is not justified.

Demeree *et al.* (1951) have noted the mutagenic activity of picric acid (2,4,6-trinitrophenol). These authors found that the number of mutations exhibited by *E. coli* exposed to 100-180 mg/ℓ of picric acid exceeded the controls by 10 fold.

f. Miscellaneous Effects

Rieder and Bukatsch (1956) reported the effect of 2,4-dinitrophenol on the glowing of luminous bacteria, a process which has been proven to be energy dependent. Treatment of a glowing culture of *Photobacterium phosphoreum* with 2,4-dinitrophenol (10^{-3} - 10^{-4} *M*) caused a rapid decrease in the intensity of light. Lower concentrations of the toxicant (10^{-5} - 10^{-6} *M*) on the other hand resulted in an increase in light intensity over control. The results of this investigation provide further support to the fact that 2,4-DNP exerts its toxicity by interfering with the energy metabolism of the cell.

2. Effect on Yeast and Fungi

The nitroaromatic compounds which have been extensively studied for their effect on yeast and fungi are 2,4-dinitrophenol and *m*-dinitrobenzene. Their toxic action on fungi and molds is evident from the fact that these compounds are well known fungistatic agents.

The effect of 2,4-DNP on nitrogen (NH_3) assimilation and respiration in the fungus *Scopulariopsis brevicaulis* has been examined by Macmillan (1956). Although 2,4-DNP failed to inhibit the endogenous oxygen uptake of the cells (in fact, it was stimulated), the compound effectively blocked the increased respiration due to ammonia, as well as the assimilation of ammonia (Table 144). The results are consistent with the view that DNP uncouples

Table 144. Effect of 2,4-Dinitrophenol on Nitrogen Assimilation and Respiration in the Fungus *Scopulariopsis brevicaulis* (Macmillan, 1956)

a. Respiration

Additions:	Oxygen consumed in 150 min. ($\mu \ell O_2$/g dry wt.)
None (Control)	27
+DNP (2.5×10^{-3} M)	47
Control + NH_3	36
+DNP (2.5×10^{-3} M)	30

b. Nitrogen Assimilation

Additions:	NH_3 Assimilated in 150 min. (mgN/g dry wt.)
Control (Energy source, 1% glucose)	16.5
+DNP (2.5×10^{-3} M)	4

oxidation from phosphorylation and thus the energy required for assimilation is not available. An additional interesting effect of DNP in the organism was that in the treated cells, the amino acids synthesized were different. For example, in the presence of DNP, the ammonia assimilated in alanine increased; the increase was accompanied with a decrease in glutamine and certain other amino acids.

Stanek and Drahonovsky (1964) reported stimulation of endogenous as well as glucose-linked respiration by nitrated phenols (2,4-dinitrophenol, 2-nitro-3,4,6-trichlorophenol and 2,3-dichloro-4-nitrophenol) in the conidia of *Neurospora sitophila* (Table 145). In agreement with the stimulation of

Table 145. Influence of Nitrophenols on the Metabolic Quotient, Oxygen Consumption and Carbon Dioxide Evolution by Conidia of *N. Sitophila* (Stanek and Drahonovsky, 1964)

Compound	Concn. (M)	Oxygen Consumption ℓ/mg/h	CO_2 Released ℓ/mg/h	Metabolic Quotient*
Endogenous				
Control (range from three experiments)	-	24.8-26.8	18.0-21.4	0.71-0.74
2,4-Dinitrophenol	10^{-4}	32.8	28.2	0.86
2-Nitro-3,4,6-chlorophenol	10^{-4}	21.8	20.2	0.93
2,3-Dichloro-4-nitrophenol	10^{-4}	11.1	17.2	1.54
Substrate - Glucose				
Control (range from three experiments)	-	58.2-71.0	18.0-21.4	0.98-1.02
2,4-Dinitrophenol	10^{-4}	84.5	95.5	1.13
2-Nitro-3,4,6-chlorophenol	10^{-4}	78.7	80.0	1.02
2,3-Dichloro-4-nitrophenol	10^{-4}	22.4	51.7	2.30

* CO_2/O_2

respiration, was the finding that the total level of carbohydrates decreased in the treated spores. Phenol derivatives, in addition, were also effective in suppressing the germination capacity of the spores; the concentration for 50% suppression being 17, 8 and 30 mg/g dry weight of spores, respectively, for 2,4-dinitro-, 2-nitro-3,4,6-trichloro-, and 2,3-dichloro-4-nitrophenol. The inhibition of germination was accompanied with changes in permeability of the cell membrane since the treated cell lost substantial quantities of cellular carbohydrates, low molecular weight phosphorus compounds (probably the degradation products of nucleic acid, etc.) and nucleic acid bases, into the incubation medium (Stanek and Drahonovsky, 1964). The chloronitrophenols, 2-nitro,3,4,6-trichlorophenol and 2,3-dichloro-4-nitrophenol possessed a stronger activity in almost all respects than the typical phosphorylation inhibitor, 2,4-dinitrophenol. Alteration of membrane permeability by nitrophenol has also been noted in bacteria.

Nitrophenolic compounds (10^{-4} M) were also reported to inhibit growth of bakers yeast Saccharomyces cerevisiae (Dedonder and Van Sumere, 1971). The test results revealed that 2,4-dinitrophenol was more inhibitory than p-nitrophenol; growth inhibition being 60% for dinitrophenol and 22% for p-nitrophenol. One tenth mM DNP was found to inhibit acetate and pyruvate oxidation and assimilation in the yeast almost completely at pH 4.8 (or less) (Stoppani, 1949;1951; Stoppani and Ramos, 1964). At a higher pH, the inhibition decreased drastically; for example, with acetate as a substrate, the inhibition by 0.1 mM DNP was 5% at pH 6.7, and none at pH 7.3. Glucose oxidation in this organism was not affected by DNP. Studies with radio-labelled acetate indicated significant changes in ^{14}C-distribution in the soluble fraction in the presence of 2,4-DNP. For instance, labeling of tricarboxylic acids diminished by 85% and similar, though much smaller, changes were observed in aspartic acid and certain dicarboxylic acids. Their findings are consistent with the conclusion that 2,4-DNP interfered with further oxidation of acetate by the Krebs Cycle.

Among nitrobenzene compounds, the metabolic effect of m-dinitrobenzene has been investigated to some extent. This compound is a potent inhibitor for molds; concentrations as low as 2.5×10^{-5} M produce complete statis of Aspergillus niger growth (Higgins, 1960). The metabolic observations indicated that growth inhibition was the result of the depression in the amino acid synthesis during the early growth phase (Higgins, 1960). At growth depressing levels of m-dinitrobenzene, no effect on oxygen utilization or glucose/oxygen ratio was evident.

3. Effect on Protozoa

Only a few studies dealing with the influence of nitroaromatic compounds on protozoa have been reported in the literature. Conner (1957) studied the effect of 2,4-dinitrophenol on the growth of ciliated protozoan Tetrahymena piriformis. The findings revealed that 2,4-DNP in the concentration range $0.5 - 1 \times 10^{-4}$ M was an effective inhibitor of growth of this organism. The ability of 2,4-DNP to act as an uncoupler of oxidative phosphorylation and to activate adenosine triphosphatase was considered to be the mechanism underlying the growth inhibition.

Picric acid was reported to be extremely toxic to an unspecified amoebae if applied to the surface at a concentration of 1% (Pollack, 1927). Hajra (1959) noted that picric acid at a concentration of 10 mg/liter was half lethal for Acanthamoeba sp., but was without effect in Neglaria gruberi. Bringman and Kuehn (1959) found the 96 hour toxicity threshold to be 900 mg/ℓ in the protozoan Microregina heterostoma. These studies revealed that indeed both di- and trinitro-substituted phenols are toxic to amoebae, but that the toxic concentration varies from species to species.

4. Effect on Unicellular Algae

In a preliminary study, Moberg et al. (1968) were able to show that 2,4-dinitrophenol at a concentration of 3×10^{-4} M inhibited the growth of Chlorella pyrenoidosa in a synchronous culture. Dedonder and Van Sumere (1971) confirmed the findings of Moberg (1968), but extended the investigation to include the effect of nitrophenols on the respiration of Chlorella. At concentrations which inhibited growth, the nitrophenols caused a stimulation of the respiration of the algae (Table 146). This stimulation of respiration is consistent with the established uncoupling action of nitrophenols. In agreement with the fact that the effectiveness of nitrophenols increases with a fall in pH (see Section III-G-1-a), it was found that stimulation was greater in magnitude at pH 5.6 than at 7.2.

Table 146. Effect of Nitrophenolic Compounds on the Growth and Respiration of Chlorella vulgaris (Dedonder and Van Sumere, 1971)

Test Compound	Concn.	% Growth Inhibition After 80 Hours	% Stimulation of Respiration, pH, 7.2 (5 Hours Incubation)
p-Nitrophenol	5×10^{-5} M	50	+30
"	1×10^{-4} M	80	+80
2,4-Dinitrophenol	5×10^{-5} M	70	0
"	1×10^{-4} M	100	20

The effect of 2,4-dinitrophenol on the uptake and metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D), a synthetic auxin, has been reported by Swets and Wedding (1964). Although the purpose of the authors in undertaking such a study was to trace the sequence of reactions of 2,4-D in the cell, the results of the investigation could also be valuable in assessing the hazards associated with the interaction of the two contaminants in the environment. For example, one contaminant may affect the toxicity, bioaccumulation and/or persistence of another contaminant in the environment. Increasing concentrations of 2,4-DNP (up to 10^{-4} M) were reported to first cause a progressive inhibition of 2,4-D uptake, but as 2,4-DNP concentration increased (up to 3.2×10^{-4} M) an increase in uptake was found (Table 147). In the absence of light or air, 2,4-DNP-linked uptake of 2,4-D was nearly doubled. The environmental consequence of the interaction may be speculated to be an increased accumulation of 2,4-D in the algae in the presence of 2,4-DNP. No information is available concerning the toxicity and persistence of 2,4-D in the presence of low concentrations of 2,4-DNP.

Table 147. Effect of 2,4-Dinitrophenol on the Uptake of 2,4-Dichlorophenoxyacetic Acid by Chlorella pyrenoidosa (Swets and Wedding, 1966)

2,4-DNP Concentration (M)	2,4-D Uptake * (μ moles/g dry wt)
None (control)	0.10
1×10^{-4}	0.03
3.2×10^{-4}	0.13
3.2×10^{-3}	0.10

* 10^{-4} M 2,4-D in the solution.

The only report concerning the influence of nitroanilines on algae is that of Villeret (1960). In this study, the effect of o-, m-, and p-nitroaniline on respiratory gas exchange of algae Chlorella vulgaris and Scenedesmus quadricauda was studied. The chemicals were found to stimulate the respiration of Chlorella in the decreasing order of o-, p-, m-nitroaniline. Similar effects were not noted in Scenedesmus. The increase in respiration in Chlorella could perhaps be due to the action of nitroaniline as an uncoupling agent similar to that of 2,4-dinitrophenol, and may not be accompanied with increased cell multiplication. The experimental data supporting or disputing this conclusion are not available.

An algae survey in the vicinity of Radford Army Ammunition Plant to determine the variations in the distribution of algae due to TNT plant effluent was carried out by Wodehouse et al. (1973). The authors noted the algae community appeared similar from station to station with only a few exceptions (for location of stations, see Figure 78). Furthermore, the distribution of taxa within major divisions of algae showed little qualitative change in response to TNT waste. For instance, at station 6A (located immediately upstream from the TNT plant), 53 taxa of algae were recorded, and at station 7A (located immediately downstream from the plant), 49 taxa were recorded. The results are, however, complexed by the fact that these stations also received other waste from industrial plants located upstream from the TNT plant. It is possible under these circumstances that one is dealing with abnormal algae population to start with, and therefore, the utility of the data in terms of the effect of TNT waste may be questionable.

5. Influence of Nitroaromatics on the Microbiological Systems Concerned With Waste Treatment

Most of the degradation in the environment and in waste treatment processes is accomplished by microorganisms. Interference with the activity of these microorganisms could result in the loss of the effectiveness of the treatment process. The following section is, therefore, devoted to the studies dealing with the effect of nitroaromatics on the activity of the microorganisms involved in purification of streams and reservoirs and waste treatment processes.

Under natural conditions, certain organic compounds, when present at low concentrations, greatly retard the processes of self-purification of reservoirs, and inhibit nitrification and the decomposition of other organic compounds (Rogovskaya, 1951; Stasiak, 1967). For example, Rogovskaya (1951) noted that 0.5 - 1.0 mg/l trinitrotoluene only slightly affected stream self-purification processes, whereas beyond 1.0 mg/l marked damaging effects on self-purification processes occurred. These results agreed with those of Ruchoft et al. (1945a) who found that concentrations of α-TNT as low as 1.17 mg/l retarded the BOD reaction.

In order to determine the effects of trinitrotoluene on sewage treatment, Enzinger (1970) set up two Bush laboratory scale bio-oxidation units. TNT was continuously pumped into the TNT test unit and the concentration was gradually increased from 12 ppm to 29 ppm; the second unit fed only synthetic sewage served as control. The monitoring of effluent COD and total carbon analysis from the two units indicated that generally the TNT test unit was less efficient in the oxidation of organic material than the control unit. That TNT retarded the biological activity was shown from the fact that oxygen uptake rates for the TNT test unit were lower than the control unit. Warburg respirometric studies undertaken to determine the effect of different levels of TNT on samples from the TNT test unit and the control unit supported the theory that TNT has adverse effects on the oxidation of organic material (Figure 80). TNT affected the respiration rates of the microorganisms from the control unit more severely than those from the TNT test unit. This suggests that the microorganisms in the TNT test unit may have been acclimated to TNT.

Contrary to the findings of Enzinger (1970), Bogatyrev (1973) noted no effect of TNT on the activated sludge process at concentrations of ≤ 50 mg/ℓ. Only a partial suppression of nitrification was observed in the presence of TNT. Mono- as well as dinitrotoluenes were also without effect on the activated sludge process.

The gross chemical composition and biochemical characteristics of activated sludge grown on a given substrate remain remarkably unaltered under normal circumstances. However, a change in the composition of the medium or perhaps even addition of toxic substances could cause a modification in the characteristics of activated sludge. This approach was used by Vaicum and Eminovici (1974) to assess toxic effects of trinitrophenol (picric acid) on activated sludge systems. A laboratory activated sludge unit was run on a nutrient feed which contained sodium acetate, urea, and phosphate. The toxicant was included in the influent following a 14 day equilibration period. The variation in respiration rate, enzyme activities (dehydrogenase and catalase) and gross activated sludge composition (glucides and total protein) were measured over an extended period of time (from 14-75 days). At the feeding level of 50 mg/l trinitrophenol, all the parameters except the

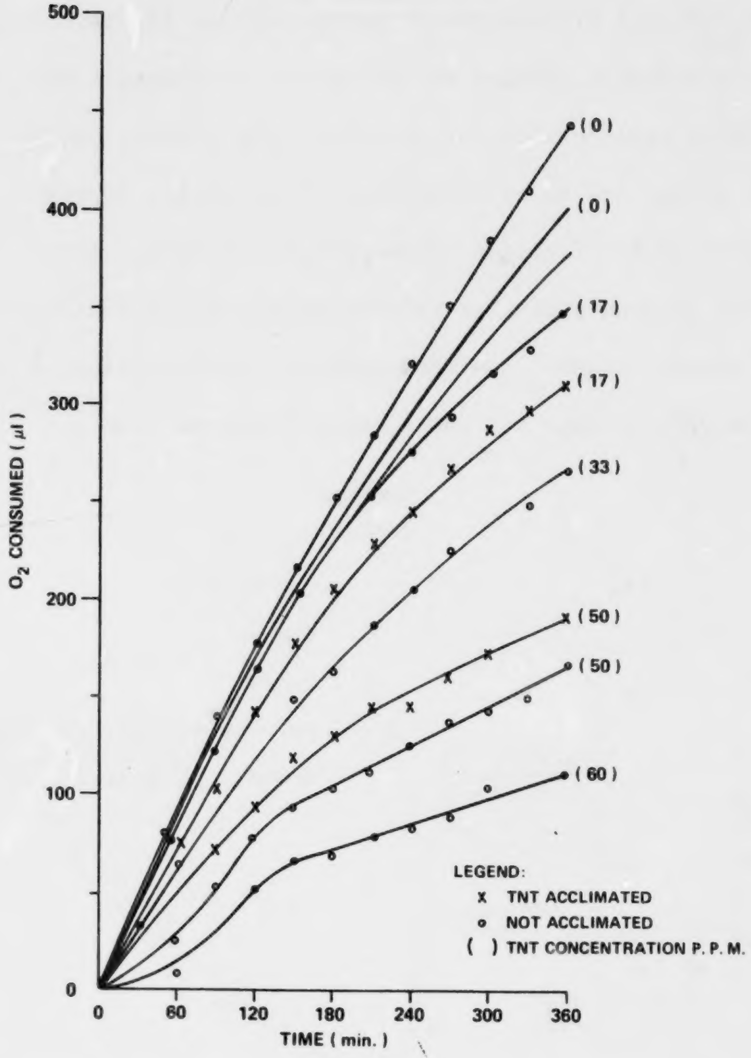

Figure 80. Respiration Rate of TNT Acclimated and Nonacclimated Microorganisms at Various Concentrations of TNT (Enzinger, 1970) (Synthetic sewage as the carbon source)

concentration of glucides showed a significant decrease after the first 24 hours. After 10 days, however, the COD removal efficiency and the sludge total protein content returned to the normal level (Figure 81). Upon the feeding of 200 mg/l trinitrophenol to the acetate fed laboratory unit, significant irreversible changes in the measured parameters were produced. After 75 days, there were no signs of recovery. The authors suggested that the effect was either due to an inhibitory effect of the substance or to the disappearance of the fraction of responsible microorganisms. The findings revealed the adverse effect of trinitrophenol on the health and activity of activated sludge systems, and consequently, on the breakdown of numerous chemicals which pass through the waste water treatment plants.

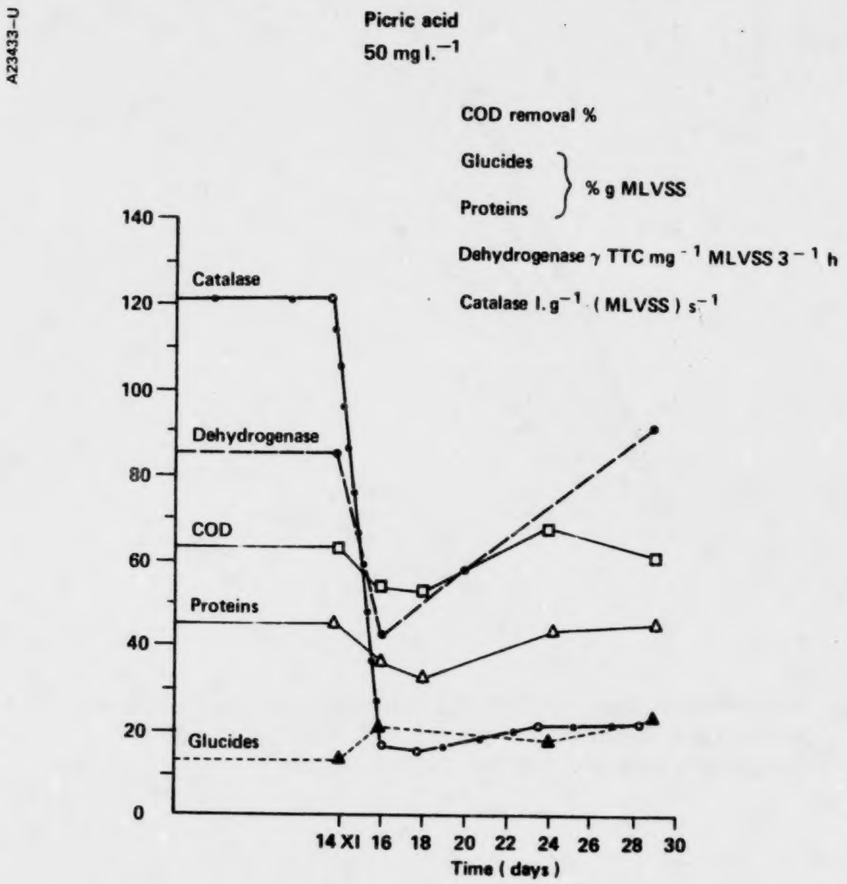

Figure 81. Effect of Trinitrophenol on the Biochemical Characteristics of Activated Sludge (Vaicum and Eminovici, 1974)

Whereas nitrotoluenes and certain nitrophenols have been shown to have an adverse effect on the microbiological systems concerned with waste treatment, and to reduce the efficiency of waste water treatment, the situation is very different with 2,4-dinitrophenol. Wilkinson (1951) has found that the presence of dinitrophenol in waste water (concentration unstated) causes an increase in population of bacteria. Shah et al. (1975) have employed the uncoupling action (ability to disconnect energy transferring sequence from the electron transfer sequence) of 2,4-dinitrophenol to stimulate microbial respiration and substrate removal in a model waste water treatment system. Experiments in a continuous reactor system with glucose as the waste (to stimulate effluents from sugar and starch industries) and *Saccharomyces cerevisiae* as the degrading organism, indicated as much as 85% increase in the degrading efficiency of glucose at 2,4-DNP concentration of 5×10^{-6} *M* (Figure 82). Cell growth on the other hand decreased in the presence of DNP; a decrease of 70% was recorded at 5×10^{-6} *M* DNP (Figure 83). The experimental data provide no information regarding the fate of DNP itself. Since the study was restricted to a model system, it cannot be said with certainty if DNP will have similar effects on specific waste-water treatment systems in the field where one is dealing with complex mixtures of chemical substances and with a variety of degrading microorganisms. Furthermore, since DNP is shown to effectively inhibit cell growth, it appears likely that upon prolonged incubation, the oxygen consumption rates will fall and may even be lower than those in the absence of DNP. Shah et al. (1975) terminated their study at the end of 24 hours and this limits the conclusion that can be drawn concerning the long range effects of this nitroaromatic on waste water treatment.

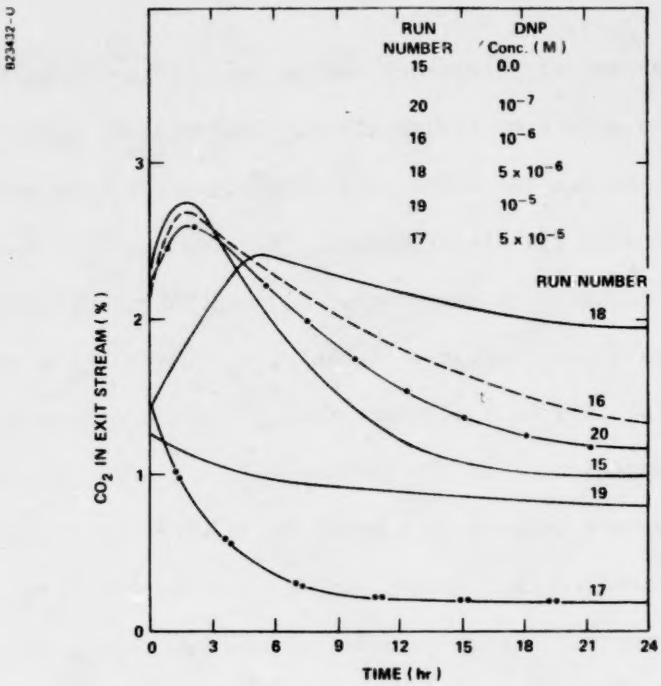

Figure 82. Concentration-Time Profiles of Carbon Dioxide in Exit Stream at Different Bulk DNP Concentrations (Shah et al., 1975)

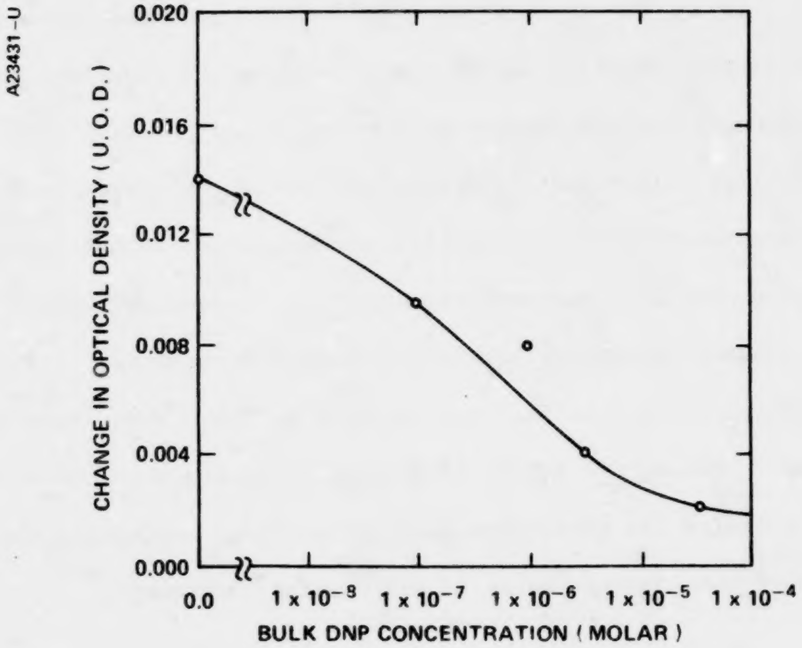

Figure 83. Effect of Bulk DNP Concentration on Cell Growth (Shah et al., 1975)

6. Effect on Natural Microbial Populations

The effect of nitroaromatic compounds (other than pesticides) on natural microbial populations of soil or aquatic systems has rarely been studied. Katznelson and Stevenson (1956) have reported results of the study in which they examined the effect of 2,4-dinitrophenol on the oxidative activity of soil. Unlike the reported effect of 2,4-DNP on pure cultures of microorganisms, the addition of 2,4-DNP (5×10^{-4} $\underline{M}$) to the natural communities of microorganisms in soil was found to completely inhibit oxygen uptake with casamino acids as substrate. Similar DNP concentrations failed to exhibit any appreciable effect on the respiration of unamended soil (Figure 84). However, no significant increase in the number of microorganisms was observed in the presence of 2,4-DNP during the period of oxidation, suggesting that the inhibitor interfered with cell multiplication. Picric acid was reported to be without effect on the microorganisms in domestic sewage (Ruchhoft and Norris, 1946). For example, the five-day BOD of domestic sewage was not lowered significantly by picric acid at 100 mg/ℓ, and was lowered only 36% at 1000 mg/ℓ. However, it must be emphasized that assessment of the toxicity of nitrophenols from the oxygen consumption data can be misleading because of the fact that nitrophenols generally stimulate oxygen consumption or leave it unaffected while inhibiting energy generation. In view of this shortcoming of the assay method, the actual toxic effect of picric acid on sewage microorganisms remains uncertain.

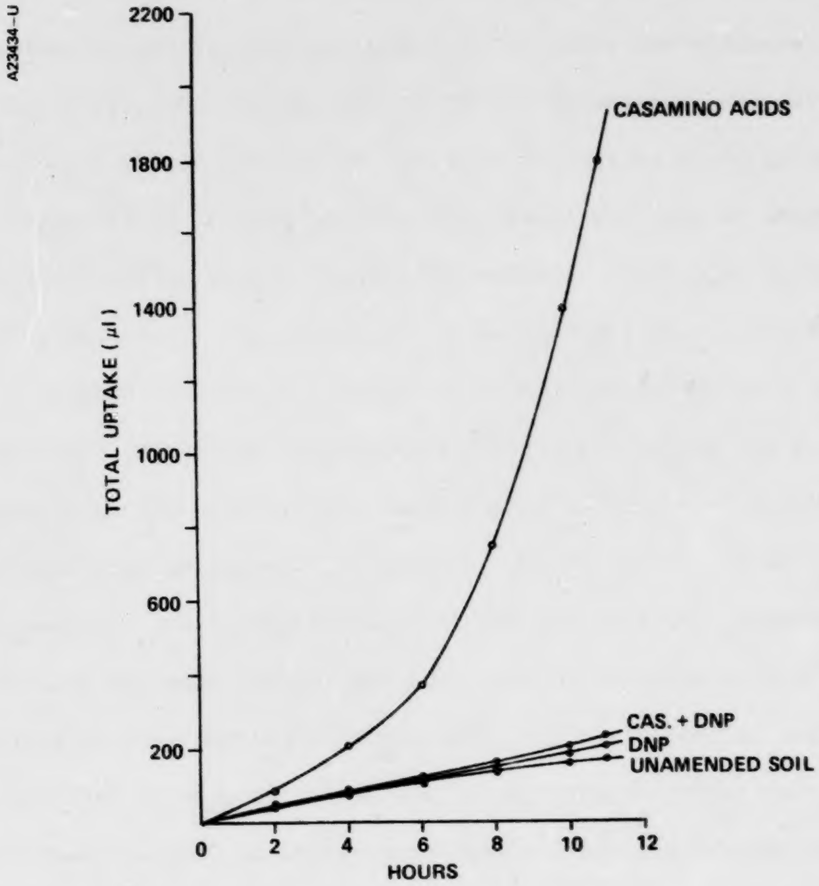

Figure 84. Inhibition of Casamino Acid Oxidation by 2,4-Dinitrophenol (Katznelson and Stevenson, 1956)

IV. Regulations and Standards

A. Current Regulation

Regulation and control over nitroaromatic chemicals is provided under several different authorities. Because this group of compounds is involved in a large number of different applications, product control at the federal level is quite varied. Effluent control, on the other hand, is exercised under basically the same authority for all the nitroaromatic compounds.

Many of the nitroaromatics with agricultural applications are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (7 U.S.C. 135-135k). This act would cover all of the nitroaromatic herbicides, fungicides, and lamprey larvicides. The major law concerning pesticides is now the Federal Environmental Pesticide Control Act of 1972, which has revised the Federal Insecticide, Fungicide, and Rodenticide Act of 1947. Under this new act, nitroaromatic chemicals with pesticide applications are required to be registered by the EPA.

Tolerances for pesticide chemical residues in or on new agricultural commodities have been established under the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 346a). Specific tolerances for a number of nitroaromatic compounds have been reported in 40 CFR 180. Among the substances listed are:

2,6-dichloro-4-nitroaniline (40CFR180.200)
2,3,5,6-tetrachloronitrobenzene (40CFR180.203)
trifluralin (40CFR180.207)
N-butyl-N-ethyl-α,α,α-trifluoro-2,6-dinitro-p-toluidine (40CRF180.208)
dinitro-p-toluidine (40CFR180.281)
pentachloronitrobenzene (40CFR180.291)
2,4-dinitro-6-octylphenyl crotonate and 2,6-dinitro-4-octylphenyl crotonate (40CFR180.341)
4,6-dinitro-o-cresol and its sodium salt (40CFR180.344)

The major federal law governing hazardous substances is the Federal Hazardous Substances Act of 1960 (15 U.S.C. 1261-1273). However, this act covers household products and toys but not the raw materials from which they are manufactured. Therefore, it does not apply directly to most of the chemicals of concern in this report.

Since several nitroaromatic compounds are used as constituents in fragrances and hair dyes, these substances would be regulated under the Federal Food, Drug, and Cosmetic Act of 1938 (21 U.S.C. 301 et seq.).

The major authority for controlling hazardous pollutants released into the environment is provided by the Federal Water Pollution Control Act (33 U.S.C. 446 et seq.) and the Clean Air Act. Both laws have been extensively amended in recent years and have set standards for air and water quality. A "sample" list of hazardous substances was issued as an advanced notice of proposed rulemaking under authority of Section 311 and Section 501 of the Federal Water Pollution Control Act as amended (33 U.S.C. 1251 et seq.) (Federal Register, 39(164):30466-30471, Aug. 22, 1974). Among the substances listed are dinitrobenzene, dinitrophenol, and nitrophenol.

The transportation of hazardous materials by rail and highway is regulated by the Hazardous Materials Control Act of 1970. This law is administered by the Hazardous Materials Regulation Board of the Department of Transportation. Air Transportation regulations are issued by the Federal Aviation Administration while the U.S. Coast Guard supervises inland and coastal water shipments. Oceanborne shipping of hazardous materials is regulated by the Federal Maritime Commission.

In January, 1974, the Department of Transportation proposed extensive changes in the rules governing the transportation of hazardous chemicals, especially by air. These changes, published in the Federal Register (January 24, 1974) include the following nitroaromatic chemicals classified as being hazardous:

dinitrobenzene
dinitrochlorobenzene
dinitrocyclohexylphenol
dinitrophenol
nitrobenzene
nitrochlorobenzene
ortho-nitroaniline
para-nitroaniline
picric acid
trinitrobenzene
trinitrobenzoic acid
trinitroresorcinol
trinitrotoluene

A code for the manufacture, transportation, storage, and use of explosive materials has been prepared by the National Fire Protection Association. This code would apply to all nitroaromatic munitions compounds.

Employee safety from hazardous chemicals is controlled by the Occupational Safety and Health Administration, established in 1971 by the Department of Labor. OSHA requires all chemical manufacturers to fill out Material Safety Data Sheets for each shipment containing chemical substances.

B. Consensus and Similar Standards

Limits have been established for maximum permissible exposure to hazardous chemicals by several agenices. Threshold limit values (TLV's) for chemicals in the workroom environment have been established by the American Conference of Governmental Industrial Hygienists. These TLV's, which are periodically revised and updated, include a number of nitroaromatic chemicals. The values given in Table 148 refer to the maximum concentration which may be present in the working environment and include the potential contribution to overall exposure by contact with the skin.

Table 148. Adopted Threshold Limit Values for Nitroaromatic Compounds (Data from American Conference of Governmental Industrial Hygienists, 1974)

Substance	ppm	mg/m^3
Dinitrobenzene (all isomers)	0.15	1
Dinitro-_o_-cresol	-	0.2
Dinitrotoluene	-	1.5
_p_-Nitroaniline	1	6
Nitrobenzene	1	5
_p_-Nitrochlorobenzene	-	1
4-Nitrobiphenyl	-	*
Nitrotoluene	5	30
Picric Acid	-	0.1
Tetryl	-	1.5
Trinitrotoluene	0.2	1.5
3,5-Dinitrotoluamide	-	5

*Human carcinogen - no assigned TLV

Exposure limits for hazardous substances have also been set by the Occupational Safety and Health Administration and the National Institute for Occupational Safety and Health. Their list of standards, which has been published in the _Federal Register_ (October 19, 1972), includes the same compounds as in Table 148, and for which the official exposure limits are the same as above.

The Manufacturing Chemists Association has sponsored the publication of _Dangerous Properties of Industrial Materials_, by Irving Sax, Reinhold Book Corp., N.Y., 1968. This compendium lists over 1,200 chemicals and their toxicity ratings, including many nitroaromatic compounds. Although the book does not provide documentation for the classification of each chemical, it is a useful guide to general toxicity information.

C. Foreign Authority

Limits for toxic substances in drinking water have been established in the U.S.S.R. (Stofen, 1973). Standards for a number of nitroaromatic chemicals are included among these values and are given below:

Substance	Limit in mg/l
Dinitrobenzene	0.5
Dinitrochlorobenzene	0.5
Dinitronaphthalene	1.0
2,4-Dinitrophenol	0.03
Nitrochlorobenzene	0.05
_m_-Nitrophenol	0.06
_o_-Nitrophenol	0.06
_p_-Nitrophenol	0.02
Trinitrotoluene	0.5

V. Summary and Conclusions

A. Summary

Commercial chemicals which fall into the category of nitroaromatics are numerous and have varying physical and chemical properties, production quantities, uses, environmental fate, and biological effects. Because of the large number of compounds studied in this report, a detailed assessment of any particular chemical was not possible. However, the conclusions discussed in this section will help develop priorities for the compounds that should receive further study and research.

Approximately 250-300 compounds are listed as commercial nitroaromatic compounds (see Chemical Index, p. 525). However, most of those compounds are produced in such small quantities that they are little more than laboratory curiosities. Nevertheless, there are approximately 40 compounds that are consumed in quantities greater than 0.5 million pounds per year, and perhaps 50-100 compounds that exceed 100,000 pounds per year. Even larger numbers of compounds fall in the 0-100,000 pound per year range.

In order to consider the relative environmental contamination potential of the individual nitroaromatic compounds, a variety of factors, which are reviewed in detail in the previous sections, must be considered. Table 149 summarizes the information that is available for most of the major commercial products. Some explanation of the table is necessary.

The 93 compounds included in Table 149 were: (1) listed as commercial products (produced in at least 1000 pounds or worth at least $1000, annually), and (2) had some environmental fate and/or biological effects information.

Table 149. Summary of Information on Nitroaromatic Chemicals

Chemical	Largest Annual Consumption (C) During (19__) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport		Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification				
2-Amino-4-nitrophenol	242 (69)	0.05	12					1,280 (Mice)		—	
4-Amino-4'-nitro-2,2'-stilbenedisulfonic acid	245 (73)	0.05	12								
2-Bromo-4,6-dinitroaniline	944 (73)	0.05	47					4,490			
2-*sec*-Butyl-4,6-dinitrophenol	3,000 (72)	1.00	3,000					25-60	+	—	96 hr. TL_m (Scud) = 1.8 mg/l
6-*tert*-Butyl-3-methyl-2,4-dinitroanisole	>119 (72)	1.00 (perfume)	119					339			
5-*tert*-Butyl-2,4,6-trinitro-*m*-xylene	>163 (70)	1.00 (perfume)	163								
1-Chloro-2,4-dinitrobenzene	6,626 (68)	0.05	331	—				500-1,593	—	+	Skin sensitizer; monitored in sulfur dye plant water effluent
2-Chloro-4-nitroaniline	2,500-3,000 (75)	0.05	150								LD (Mice) = 500 mg/kg I.P.

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19__) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
4-Chloro-2-nitroaniline	503 (67)	0.05	25								LD_{50} (Mice) = 63 mg/kg I.V.
1-Chloro-3-nitrobenzene	7,908 (66)	0.05	395		>64	27	307	555	—	+	Monitored in river, drinking, and waste water
1-Chloro-2-nitrobenzene	60,000 (75)	0.05	3,000	—	>64	22	227	288	—	+	Monitored in river and waste water
1-Chloro-4-nitrobenzene	110,000 (75)	0.05	5,500	—	>64	27	224	420	—	+	Monitored in waste water
4-Chloro-3-nitrobenzene-sulfonamide	743 (73)	0.05	37								
2-Chloro-5-nitrobenzene-sulfonic acid and sodium salt	500-600 (75)	0.05	30								
4-Chloro-3-nitrobenzene sulfonic acid	174 (68)	0.05	9								
4-Chloro-3-nitrobenzene-sulfonyl chloride	345 (71)	0.05	17								
2-Chloro-4-nitrobenzoic acid		0.05		+							

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19___) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
o-(4-Chloro-3-nitrobenzoyl)benzoic acid	147 (67)	0.05	7								
2-Chloro-4-nitrotoluene		0.05					3,020				
2-Chloro-6-nitrotoluene		0.05									Mild skin irritant
4-Chloro-2-nitrotoluene	693 (63)	0.05	35								
4-Chloro-3-nitrotoluene	102 (66)	0.05	5								
2,6-Dichloro-4-nitroaniline	607 (66)	0.05 (same use as fungicide)	30					418- >5,000	+	+	96 hr. TL_m (Bluegills) = 37 ppm; produced tumors
1,2-Dichloro-4-nitrobenzene	3,000-3,600 (75)	0.05	180				579	643			
1,4-Dichloro-2-nitrobenzene	700-800 (75)	0.05	40				686	1,210			
2,5-Dichloro-3-nitrobenzoic acid		0.05						3,500			

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19__) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport		Oral LD$_{50}$ (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification				
2,4-Dichlorophenyl-4-nitrophenyl ether (Nitrofen)		1.00						3,050			
O,O-Diethyl-o,p-nitrophenylphosphorothioate (Ethyl parathion)	15,259 (70)	1.00	15,259					3.6-13			48 hr. TL$_m$ (Bluegills) = 47 mg/l
O,O-Dimethyl-o,p-nitrophenylphosphorothioate (Methyl parathion)	48,890 (73)	1.00	48,890					9-25			
2,4-Dinitroaniline	>679 (72)	0.05	34					1,800	+		
p-(2,4-Dinitroanilino)-phenol	33 (64)	0.05	2								
2,4-Dinitroanisole		0.05							+		Non-tumorigenic; LD (Rats) = 100 mg/kg oral
3',4-Dinitrobenzanilide	16 (69)	0.05	1								
1,3-Dinitrobenzene	12,000 (72)	0.05	600	+	>64	8			—	+	Monitored in drinking water

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19___) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport		Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification				
3,5-Dinitrobenzoic acid	500 (75)	0.05	25	—							
4,4'-Dinitrobiphenyl		0.05									Produced neoplasms
Dinitrobutylphenol, ammonium salt	58 (67)	1.00	58					45			
Dinitrocaprylphenyl crotonate		1.00						980-1,190	+	—	48 hr. TL_m (Harlequin Fish) = 0.27 ppm
4,6-Dinitro-o-cresol	>218 (72)	1.00	218	+ + +				10-50	+	—	Non-tumorigenic; 48 hr. TL_m (Rainbow Trout) = 210 mg/1; monitored in chemical plant effluent
2,4-Dinitro-α-naphthol		0.05							+	—	LD (Dog) = 30 mg/kg I.V.
2,4-Dinitrophenol	1,000 (75)	0.05	50	±		8		30	+	—	Non-tumorigenic; LC_{min} (Fish) = 0.5-38 mg/1
4,4'-Dinitrostilbene-2,2'-disulfonic acid	9,858 (72)	0.05	493								
3,5-Dinitrotoluamide		0.05						560	—	—	

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19__) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport		Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification				
2,4-(and 2,6-)Dinitrotoluene	471,237 (73)	0.05	23,561	+ +			•	268-707	—	+	Non-tumorigenic; 96 hr. TL_m (Bluegills) = 16 mg/l; monitored in drinking water and waste water
1-Fluoro-2,4-dinitrobenzene									—		Skin sensitizer; tumor promoter LD (Rats) = 50 mg/kg oral
p-Fluoronitrobenzene											LD (Rats) = 250 mg/kg oral
4-(Methylsulfonyl)-2,6-dinitro-N,N-diphenylaniline (Nitralin)		1.00						>2,000			
3'-Nitroacetanilide	15 (70)	0.05	1								
3'-Nitroacetophenone								3,250		+	
o-Nitroaniline	6,00C (75)	0.05	300	+ +	>64	12		535-3,520	—	+	
m-Nitroaniline	>192 (73)	0.05	10	+ +	>64	8		535-900	—	+	
p-Nitroaniline	14,000 (75)	0.05	700	+ + +	>64	6		1,410-3,249	—	+	

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19___) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
4-Nitro-o-anisidine (1-amino)	>345 (73)	0.05	17								
5-Nitro-o-anisidine (1-amino)	>127 (67)	0.05	6					704			
o-Nitroanisole	2,500-3,500 (75)	0.05	150		>64					+	
p-Nitroanisole	750-1,500 (75)	0.05	75		>64					+	
p-Nitrobenzaldehyde										+	LD_{50} (Rats) = 545 mg/kg I.P.
Nitrobenzene	655,000 (74)	0.05	32,750	±	>64	13	79	640-664	—	+	Monitored in waste and drinking water and a chemical plant lagoon
m-Nitrobenzenesulfonic acid and sodium salt	3,654 (70)	0.05	183								
m-Nitrobenzenesulfonyl chloride	23 (63)	0.05	1								
o-Nitrobenzoic acid				+ + +	8	7			—		LD_{50} (Mice) = 3,100 mg/kg I.P.
m-Nitrobenzoic acid and sodium salt	911 (69)	0.05	46	±	>64	11		1,820	—		Non-tumorigenic

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19__) (Import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
p-Nitrobenzoic acid		0.05		+ + +	4	13		1,960	—		
o-Nitrobiphenyl		1.00						1,230	—	—	Non-tumorigenic
4-Nitrodiphenylamine	>460 (72)	0.05	23								
1-Nitronaphthalene	6,290 (72)	0.05	315					120	—	+	
3-Nitro-1,5-naphthalene-disulfonic acid	223 (65)	0.05	11								
7-(and 8-)Nitronaphth-(1,2-d)(1,2,3)oxadiazole-5-sulfonic acid	551 (69)	0.05	28								
o-Nitrophenol	10,000-15,000 (75)	0.05	750	±	>64	12	3	2,828	—	+	Non-tumorigenic; 48 hr. TL_m (Bluegills) = 46.3-51.6; monitored in chemical plant lagoon
p-Nitrophenol and sodium salt	60,000-100,000 (75)	0.05	5,000	+ + +	16	14	43	350-467	±	+	Non-tumorigenic; monitored in parathion plant water effluent
4'-(p-Nitrophenyl) acetophenone	42 (70)	0.05	2								

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19__) (Import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport		Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification				
2-Nitro-p-phenylene-diamine		1.00 (hair dye)									Carcinogenic
4-Nitro-o-phenylene-diamine		1.00 (hair dye)									Carcinogenic
4-Nitrostilbene											Carcinogenic; LD_{100} (Mice) = 500 mg/kg I.P.
2-Nitrotoluene	10,000-12,000 (75)	0.05	600		>64	23	7	891	—	+	6 hr. LC_{min} (Fish) ≤ 18-40 mg/l; monitored in TNT plant water effluent
3-Nitrotoluene		0.05			>64	28	9	1,072-2,282	—	+	6 hr. LC_{min} (Fish) = 14-30 mg/l
4-Nitrotoluene	17,750 (68)	0.05	888	±	>64	26		2,144	—	+	6 hr. LC_{min} (Fish) = 20-25 mg/l; monitored in TNT plant water effluent
5-Nitro-o-toluenesulfonic acid (1-SO_3H)	7,955	0.05	398								
3-Nitro-p-toluenesulfonic acid (1-SO_3H)	81 (68)	0.05	4								

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19___) (import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
2-Nitro-p-toluidine (1-amino)	864 (67)	0.05	43								
4-Nitro-o-toluidine (1-amino)	>334 (72)	0.05	17								
5-Nitro-o-toluidine (1-amino)	353 (73)	0.05	18					574			
Nitroxylenes	545 (59)	0.05	27								
p-Nitro-o-xylene	>407 (72)	0.05	20								
Pentachloronitrobenzene	3,000 (72)	1.00	3,000				25,520	1,650-1,740	—	—	Carcinogenic
2,3,5,6-Tetrachloronitrobenzene		1.00					4,074				Produced neoplasms
2,3,4,6-Tetranitroaniline											LD_{min} (Dogs) = 2,500 mg/kg S.C.
N,2,4,6-Tetranitroaniline (Tetryl)	3,600 (73) (discontinued)	0.05			—				—	—	Produced neoplasms
1,2,4-Trichloro-5-nitrobenzene							1,555				LD_{50} (Blackbirds) = 100 mg/kg oral

Table 149. Summary of Information on Nitroaromatic Chemicals (Cont'd)

Chemical	Largest Annual Consumption (C) During (19___) (Import quantities sometimes used) (thousands of pounds)	Release Factor (RF) Pesticides and perfumes=1.00; Explosives=0.05; Chemical intermediates=0.05	Contamination Factor (C x RF)	Biodegradability		Transport					
				Summary	Days to Disappearance	Bioconcentration	Ecological Magnification	Oral LD_{50} (Rats) (mg/kg)	Metabolic Effects	Hematologic Effects	Remarks
α,α,α-Trifluoro-2,6-dinitro-N,N-dypropyl-p-toluidine (Trifluralin)	25,500 (72)	1.00	**25,500**					5,000			48 hr. TL_m (Trout) = 11 mg/1
2,4,6-Trinitrophenol		0.05		±					—	—	
2,4,6-Trinitrotoluene	432,000 (73)	0.05	21,600	+					—	+	Non-tumorigenic; 96 hr. TL_m (Bluegills) = 2.6 mg/1; monitored in TNT plant effluent
2,4,6-Trinitroresorcinol		0.05		—							

Commercial compounds having no data on fate or effects were selected if available information indicated that they were produced or imported (the sign, >, was used when import data were used) in quantities exceeding 100,000 pounds per year. Unfortunately, the chemical marketing literature is not comprehensive enough to assure that all nitroaromatic compounds consumed in over 100,000 pounds are included. However, numerous manufacturers were personally contacted in an effort to identify all nitroaromatics consumed in over 500,000 pounds per year. Thus, the list is fairly comprehensive for nitroaromatics in that consumption category.

Nitroaromatic compounds find applications as pesticides, perfumes, explosives, and chemical intermediates. Each of these uses has a different potential for release of the compound to the environment. The release factor, which was calculated in Table 149, was used to convert the consumption figure into a number related to the quantities likely to be released to the environment. Pesticides and perfumes were assigned a factor of 1.00, since they would be used in such a way that 100% would be released to the environment or would come into human contact. Chemical intermediates were given a small factor since most of the chemicals will be converted to another material (0.05 is arbitrary, but is probably an upper limit of the amount that might be released during production, transport, and use). Even though a high release factor was used, there are only about six non-pesticidal chemicals that are produced in large enough quantities that the amount likely to be released to the environment would exceed the quantity from a relatively low volume pesticide ($\sim$ 1 million pounds per year).

An attempt was made to include the biodegradation data in the contamination factor calculation. However, after examining the information, it did not appear that a quantitative value should be assigned. Thus, the summary uses only qualitative signs of biodegradability (see Table 44 for the key). Also, much of the biodegradation work was done with different pure cultures of microorganisms and correlation of the results to environmental fate is difficult. One study by Alexander and Lustigman (1966) did evaluate the biodegradability of a number of disubstituted nitroaromatics with a mixed population of microorganisms. These results are also included in Table 149 (the days noted correspond to the time necessary for the ultraviolet adsorbancy to return to the value of the blank). In general, nitroaromatics appear to be fairly stable, although, depending upon the other substituents, some chemicals do biodegrade. On the other hand, some nitroaromatics (e.g., chloronitrobenzenes) are extremely persistent. With the exception of TNT, not enough information is available to determine the environmental photodegradation potential for the nitroaromatics. However, most of the chemicals absorb ultraviolet light at wavelengths greater than 290 nm (wavelengths available in sunlight).

The bioconcentration and ecological magnification potential have been calculated for a number of compounds included in Table 149. With the exception of the highly chlorinated nitrobenzenes, which are mostly used as pesticides, none of the compounds is likely to bioaccumulate in higher trophic levels to the same extent as many of the organochlorine pesticides (for comparison, the bioconcentration of endrin was 2,953; ecological magnification for DDT was 16,950). However, increases in the calculated concentration of several hundred are not uncommon.

Many of the important commercial nitroaromatic compounds have been detected in drinking water, river water, and waste water effluents (see remarks, Table 149). Detecting a chemical in the environment is an important factor in determining its potential hazard. However, lack of detection cannot be interpreted to indicate that a chemical is not an environmental contaminant unless a very sensitive and specific analytical method is used with a well-designed sampling scheme. This is rarely the case, as evidenced by the fact that no nitroaromatic compounds have been detected in effluent or ambient air samples, even though the high vapor pressure of some of the chemicals would suggest substantial evaporation. Several non-commercial nitroaromatic compounds have been detected in water samples (see Table 150). Most of these chemicals are by-products from TNT or DNT plants (with 4,6-dinitro-2-aminophenol, the source is unknown). Other nitration processes may produce by-products which could be emitted to the environment, but little information on identity or quantities lost is available.

Table 150. Nitroaromatic Compounds Detected in River, Drinking, or Waste Waters That Are Not Commercial Products

Chemical	Type of Sample	Concentration
4,6-Dinitro-2-aminophenol	Drinking water	
2,4-Dinitrotoluene-5-sulfonic acid	TNT plant water effluent	
2,4-Dinitrotoluene-3-sulfonic acid	TNT plant water effluent	
3,5-Dinitrobenzenesulfonic acid	TNT plant water effluent	
Trinitrobenzoic acid	TNT plant water effluent	0.80 ppm
3,4-Dinitrotoluene	Explosives (DNT) plant water effluent	40 ppm

Nitroaromatic compounds exhibit several distinct and important biological effects. Nitrobenzene and its derivatives (dinitrobenzenes, nitrotoluenes) primarily affect the hematologic system through the production of methemoglobinemia, sulfhemoglobinemia, Heinz bodies, and red cell destruction. 2,4-Dinitrophenol and related structures (2-sec-butyl-4,6-dinitrophenol, 4,6-dinitro-o-cresol) are unique in their ability to "uncouple" oxidative phosphorylation by suppressing the coupling of electron flow to synthesis of ATP. This uncoupling effect produces a profound disturbance of metabolic function. In addition, a number of nitroaromatic chemicals (most of which are not produced in large commercial quantities) are active tumor-producing agents in animals.

Repeated exposures to nitroaromatic chemicals can typically result in irreversible damage to the major organs responsible for foreign compound detoxification (principally the liver and kidneys). Numerous fatalities have occurred in humans as a result of occupational and accidental poisoning by nitroaromatic compounds. Non-fatal exposure to these substances has produced a plethora of cases involving cyanosis, anemia, CNS disturbance, cataracts, liver disease, allergic reactions, and severe contact dermatitis. In most instances of acute exposure, however, the resulting effects on the hematologic system or metabolic function are rapidly reversible following removal from exposure. In only a few cases (e.g., 4,6-dinitro-o-cresol, m-dinitrobenzene) does it appear that cumulative toxicity may occur from long-term, low-level exposures.

In general, the dinitrophenol derivatives are far more acutely toxic than the nitrobenzene compounds (rat oral LD_{50} < 75 mg/kg) and the danger from single exposures may be very great. Most of the nitroaromatic compounds which have been tested are readily absorbed by oral, dermal, and inhalational routes. Increasing substitution of the nitroaromatic nucleus, as well as the addition of bulky substituents, tends to decrease both absorption and toxicity. In lower animals such as fish, the nitroaromatics are comparatively quite highly toxic, with nitrophenol derivatives being particularly active.

A number of nitroaromatic chemicals are active tumor-initiators, tumor-promoters, or complete carcinogens in animals (Table 151). These compounds appear to owe their tumorigenic activity to a highly reactive metabolic intermediate, believed to be a nitroso or hydroxylamino derivative. These same intermediates are formed during the metabolic conversion of the classical aromatic amine carcinogens. Thus, it has been possible to demonstrate that most nitro analogs of aromatic amine carcinogens are likewise active tumor-producing agents. Moreover, it is known that certain heterocyclic nitro compounds and polychlorinated nitrobenzenes possess unique tumorigenic properties. It is important to note, however, that while many nitroaromatic compounds produce tumors in animals, these neoplastic growths are not necessarily malignant. The information presented in Table 151 distinguishes between compounds that produced neoplasms (not necessarily malignant) and those that induced malignant carcinomas.

It is exceedingly difficult to assess the overall toxic hazard posed by many of the commercially significant nitroaromatic chemicals. The published literature is sorely deficient in data regarding chronic exposures, mutagenic and teratogenic effects, and possible carcinogenic bioactivation for many of the high-volume nitroaromatics produced today. The priorities of past toxicology research with the nitroaromatics have generally not emphasized those compounds with highest production volume or greatest environmental contamination potential.

Table 151. Tumor Production in Animals by Nitroaromatic Chemicals

Compound	Effect
2-Amino-4-(p-nitrophenyl)thiazole	carcinoma
1-Chloro-2,4-dinitronaphthalene	carcinoma
2,6-Dichloro-4-nitroaniline	neoplasm
1,2-Dichloro-3-nitronaphthalene	neoplasm
4,4'-Dinitrobiphenyl	neoplasm
2,5-Dinitrofluorene	carcinoma
2,7-Dinitrofluorene	carcinoma
1-Fluoro-2,4-dinitrobenzene	tumor promotion
Hexanitrodiphenylamine	neoplasm
2-Hydrazino-4-(p-nitrophenyl)thiazole	carcinoma
p-Nitrobiphenyl	carcinoma
2-Nitrofluorene	carcinoma
5-Nitro-2-furaldehyde semicarbazone	carcinoma
N-[4-(5-Nitro-2-furyl)-2-thiazolyl]acetamide	carcinoma
2-Nitronaphthalene	neoplasm
p-Nitroperbenzoic acid	neoplasm
4-Nitro-o-phenylenediamine	carcinoma
2-Nitro-p-phenylenediamine	carcinoma
4-Nitroquinoline-N-oxide	carcinoma
p-Nitrostilbene	carcinoma
Pentachloronitrobenzene	carcinoma
2,3,4,5-, 2,3,4,6-, and 2,3,5,6-Tetrachloronitrobenzene	neoplasm

B. Conclusions

This report has focused on non-pesticidal nitroaromatic compounds. Most of the commercial products are consumed as chemical intermediates and, therefore, the major source of environmental contamination is from chemical production or use plants. However, with the exception of TNT production and use, information on the quantities of chemicals released is not available. Many of the chemicals appear to be persistent and some may bioaccumulate slightly. The toxicologic information is not adequate, considering the quantities of some compounds that are produced. Few of the commercially important compounds have been tested for carcinogenic, mutagenic, or teratogenic effects. Nevertheless, an attempt has been made to determine the relative environmental hazard of the non-pesticidal nitroaromatic compounds (Table 152).

The compounds in Table 152 are listed in descending order by the value of the contamination factor (0.05 x consumption) calculated in Table 149. However, the order is not necessarily the same as for pollution potential, although an exact ordering based upon the available information does not seem justified. Nevertheless, some of the compounds appear to have high priorities for further study. All the nitroaromatic compounds that have been detected in drinking water should be closely examined and tested. Detection in drinking water is the best evidence that the chemical is persistent, at least long enough to be transported from the chemical plant to the drinking water plant. Air monitoring studies, which unfortunately are not available, would have been extremely useful in setting priorities.

The monochloronitrobenzene group is interesting in that the one compound produced in the smallest quantity (1-chloro-3-nitrobenzene) is the only one detected in drinking water. Many explanations are possible, including:

Table 152. Nitroaromatic Compounds Which Have a High Potential for Being Environmental Pollutants

Chemical	Contamination Factor	Biodegradable	Monitored in Water Effluents or River Water	Monitored in Drinking Water	Oral LD_{50} (rats) mg/kg	Fish Toxicity (96 TL_M; mg/l)	Tested for Carcinogenicity
Nitrobenzene	32750	?	Yes	Yes	640-664	--	No
Dinitrotoluene	23561	Yes	Yes	Yes	268-707	16	Yes-Neg.
2,4,6-Trinitrotoluene (TNT)	21600	Yes	Yes	No	--	2.6	Yes-Neg.
1-Chloro-4-nitrobenzene	5500	No	Yes	No	420	--	No
p-Nitrophenol	5000	Yes	Yes	No	350-467	--	Yes-Neg.
1-Chloro-2-nitrobenzene	3000	No	Yes	No	288	--	No
4-Nitrotoluene	888	?	Yes	No	2144	20-50 (6 hr. LC_{MIN})	No
o-Nitrophenol	750	?	Yes	No	2828	46.3-51.6 (48 hr. TL_M)	Yes-Neg.
p-Nitroaniline	700	?	No	No	1410-3249	--	No

Table 152. Nitroaromatic Compounds Which Have a High Potential for Being Environmental Pollutants (Cont'd)

Chemical	Contamination Factor	Biodegradable	Monitored in Water Effluents or River Water	Monitored in Drinking Water	Oral LD_{50} (rats) mg/kg	Fish Toxicity (96 TL_M; mg/l)	Tested for Carcino-genicity
2-Nitrotoluene	600	No	Yes	No	891	18-40 (LC_{MIN})	No
1,3-Dinitrobenzene	600	No	Yes	Yes	--	--	No
4,4'-Dinitrostilbene-2,2'-disulfonic acid	493	--	No	No	--	--	No
5-Nitro-*o*-toluenesulfonic acid	398	--	No	No	--	--	No
1-Chloro-3-nitrobenzene	395	No	Yes	Yes	555	--	No
1-Chloro-2,4-dinitrobenzene	331	No	Yes	No	500-1593	--	No
1-Nitronaphthalene	315	--	No	No	120	--	No
o-Nitroaniline	300	?	No	No	535-3520	--	No
m-Nitrobenzenesulfonic acid	183	--	No	No	--	--	No

Table 152. Nitroaromatic Compounds Which Have a High Potential for Being Environmental Pollutants (Cont'd)

Chemical	Contamination Factor	Biodegradable	Monitored in Water Effluents or River Water	Monitored in Drinking Water	Oral LD_{50} (rats) mg/kg	Fish Toxicity (96 TL_M; mg/l)	Tested for Carcinogenicity
1,2-Dichloro-4-nitrobenzene	180	--	No	No	643	--	No
6-tert-Butyl-2,4,6-trinitro-m-xylene	163	--	No	No	--	--	No
2-Chloro-4-nitroaniline	150	--	No	No	--	--	No
o-Nitroanisole	150	No	No	Yes*	--	--	No
6-tert-Butyl-3-methyl-2,4-dinitroanisole	119	--	No	No	339	--	No
p-Nitroanisole	75	No	No	Yes*	--	--	No
2,4-Dinitrophenol	50	?	No	No	30	0.5-38 (LC_{MIN})	Yes-Neg.
2-Bromo-4,6-dinitroaniline	47	--	No	No	4490	--	No
m-Nitrobenzoic acid	46	?	No	No	1820	--	Yes-Neg.
2-Nitro-p-toluidine	43	--	No	No	--	--	No

* Nitroanisole has been removed from EPA's list of organic chemicals identified in drinking water.

Table 152. Nitroaromatic Compounds Which Have a High Potential for Being Environmental Pollutants (Cont'd)

Chemical	Contamination Factor	Biodegradable	Monitored in Water Effluents or River Water	Monitored in Drinking Water	Oral LD_{50} (rats) mg/kg	Fish Toxicity (96 TL_M; mg/1)	Tested for Carcinogenicity
1,4-Dichloro-2-nitrobenzene	40	--	No	No	1210	--	No
4-Chloro-3-nitrobenzenesulfonamide	37	--	No	No	--	--	No
4-Chloro-2-nitrotoluene	35	--	No	No	--	--	No
2,4-Dinitroaniline	34	--	No	No	1800	--	No
2,6-Dichloro-4-nitroaniline	30	--	No	No	418- >5000	37	Yes; produced tumors
2-Chloro-5-nitrobenzenesulfonic acid	30	--	No	No	--	--	No
7-(and 8-)Nitronaphth(1,2-d)(1,2,3)oxadiazole-5-sulfonic acid	28	--	No	No	--	--	No
Nitroxylenes	27	--	No	No	--	--	No
4-Chloro-2-nitroaniline	25	--	No	No	--	--	No

1) the meta-isomer may be more stable in the environment (the fact that the ortho-isomer was detected after traveling 1000 miles in the Mississippi River does not support this explanation) and 2) the meta-isomer may be an undesirable by-product and, therefore, only minimal efforts are exerted to recover the chemical when produced. The chloronitrobenzene compounds in general appear to be very persistent and, therefore, even the compounds produced in small quantities should be studied. 1-Chloro-2,4-dinitrobenzene, in particular, should be examined further because it is such a potent skin sensitizer.

p-Nitrophenol is another compound that is produced in large enough quantities that sizable amounts might be released to the environment. However, since p-nitrophenol is a major breakdown product of the parathions and nearly 50-60 million pounds of parathions are intentionally released to the environment each year, it seems unlikely that losses of p-nitrophenol from product and chemical intermediate use would be significant compared to the parathion-derived source.

There is one compound, 4,6-dinitro-2-aminophenol, that has been detected in drinking water but is not an important commercial product or an obvious by-product of a large commercial product. It seems likely that the precursor to the dinitroaminophenol probably contains three nitrogen substituents. This limits possibilities for its source considerably, with prime candidates being the dinitroaniline herbicides or TNT.

Although only a few of the compounds have been tested for carcinogenic activity, one compound, 2,6-dichloro-4-nitroaniline, has been found to be tumorigenic. The low production volume suggests that this compound may pose an occupational or localized environmental problem, but is not likely to be a widespread environmental contaminant. However, the compound is also used as a fungicide which will result in considerable release to the environment and potential human exposure to the chemical.

1-Nitronaphthalene may be a significant environmental contaminant since it is related by reduction to α-naphthylamine, a suspected carcinogen. For this reason, study of the effluents from the one plant (DuPont) that produces the compound seems desirable.

In conclusion, it appears that a sizable number of nitroaromatic compounds are produced in large enough quantities that significant quantities can be released to the environment, even though most of the chemicals are used as chemical intermediates. Detection of these chemicals in drinking water supports this contention. Air monitoring and further water monitoring studies to more accurately determine the extent of nitroaromatic environmental contamination would seem desirable. Many of the chemicals are stable in the environment, and some seem to be extremely persistent. Most of the biological effects that have been noted are reversible and have no-effect concentrations above likely environmental concentrations. However, *in vitro* screening of these compounds for mutagenic/carcinogenic effects using inexpensive methods, such as devised by Ames *et al*. (1975), would appear to be very desirable.

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Dow Chem.   Midland, MI
Vicksburg   Vicksburg, MS

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GAF   Rensselaer, NY

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GAF   Rensselaer, NY

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Dupont   Deepwater, NJ

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GAF   Rensselaer, NY

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Martin Marietta   Sodyeco, NC

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Toms River   Toms River, NJ

2-Chloro-3,5-dinitrobenzoic acid
Ashland   Great Meadows, NJ

4-Chloro-3,5-dinitrobenzoic acid
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GAF   Rensselaer, NY

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Olin   Rochester, NY

4-Chloro-N-isopropyl-3-nitrobenzene sulfonamide
Toms River   Toms River, NJ

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Toms River   Toms River, NJ

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Toms River   Toms River, NJ

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Frank Enterprises	Columbus, OH

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Dupont	Deepwater, NJ

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Olin	Rochester, NY

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American Color and Chem.	Lock Haven, PA
Mobay	Bayonne, NJ Charleston, SC
Dupont	Deepwater, NJ

4-Chloro-3-nitroaniline (3-Nitro-4-chloroaniline) (p. 4, 19, 54, 351)

Frank Enterprises	Columbus, OH
Olin	Rochester, NY

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2-Chloro-4-nitrobenzamide

Salsbury Labs	Charles City, IA

4'-Chloro-3-nitrobenzanilide (p. 446)

1-Chloro-2-nitrobenzene (o-Chloronitrobenzene; 2-Chloronitrobenzene) (p. 3, 5, 20, 24, 26, 48, 53, 54, 58, 61, 68, 71, 73, 74, 77, 80, 104, 122, 123, 124, 128, 129, 135, 139, 140, 182, 188, 191, 193, 225, 226, 227, 261, 280, 294, 295, 356, 389, 390, 401, 495, 497

American Color and Chem.	Lock Haven, PA
Dupont	Deepwater, NJ
Monsanto	Sauget, IL

1-Chloro-3-nitrobenzene (3-Chloronitrobenzene; m-Chloronitrobenzene) (p. 3, 5, 48, 53, 59, 62, 68, 71, 73, 82, 122, 124, 128, 129, 191, 193, 225, 226, 227, 356, 495, 497)

GAF	Linden, NJ

1-Chloro-4-nitrobenzene (p-Chloronitrobenzene; 4-Chloronitrobenzene) (p. 3, 5, 20, 26, 33, 48, 53, 57, 58, 61, 68, 71, 73, 77, 78, 79, 80, 98, 101, 104, 122, 124, 129, 135, 139, 182, 191, 193, 225, 226, 227, 257, 258, 261, 280, 282, 294, 295, 356, 357, 380, 381, 389, 390, 495, 496, 497)

Dupont	Deepwater, NJ
Monsanto	Sauget, IL

2-Chloro-5-nitrobenzenesulfinic acid

Toms River	Toms River, NJ

4-Chloro-3-nitrobenzenesulfonamide (p. 5, 52, 60, 64, 83)

GAF	Rensselaer, NY
Inmont	Hawthorne, NJ
Nyanza	Ashland, MA
Salsbury Labs	Charles City, IA
Toms River	Toms River, NJ

4-Chloro-3-nitrobenzenesulfonanilide

Toms River	Toms River, NJ

2-Chloro-4-nitrobenzenesulfonic acid (p. 5)

2-Chloro-5-nitrobenzenesulfonic acid (p. 5, 51, 60, 64, 84)

Dupont	Deepwater, NJ
Toms River	Toms River, NJ
Nyanza	Ashland, MA

2-Chloro-5-nitrobenzenesulfonic acid, sodium salt (p. 6, 20, 51, 60, 64, 84)

Dupont	Deepwater, NJ

4-Chloro-3-nitrobenzenesulfonic acid (p. 5, 51, 90)

GAF	Rensselaer, NY
Toms River	Toms River, NJ

4-Chloro-3-nitrobenzenesulfonic acid, potassium salt
  Allied Chem. Buffalo, NY

4-Chloro-3-nitrobenzenesulfonyl chloride (p. 6, 52, 90)

2-Chloro-4-nitrobenzoic acid (2-Chloro-4-nitrobenzoate) (p. 6, 55, 90, 142, 143, 151, 152, 182)
  Bofors Indust. Linden, NJ
  RSA Corp. Ardsley, NY
  Salsbury Labs Charles City, IA

2-Chloro-5-nitrobenzoic acid (p. 90)
  Toms River Toms River, NJ

4-Chloro-3-nitrobenzoic acid (p. 55)
  Ashland Great Meadows, NJ

5-Chloro-2-nitrobenzoic acid (p. 90)

2-Chloro-5-nitrobenzotrifluoride (p. 90)

4-Chloro-5-nitrobenzotrifluoride (4-Chloro-α,α,α-trifluoro-3-nitrotoluene) (p. 55, 90)
  GAF Rensselaer, NY
  Olin Rochester, NY

2-Chloro-4-nitrobenzoyl chloride
  Bofors Linden, NJ

o-(4-Chloro-3-nitrobenzoyl)benzoic acid (p. 6, 51, 90)
  American Color and Chem. Lock Haven, PA
  GAF Linden, NJ

2-Chloro-4-nitrophenol (p. 55, 155, 158, 182, 227, 334, 358, 442)

2-Chloro-5-nitrophenol (p. 227, 440)

3-Chloro-2-nitrophenol (p. 227, 442)

3-Chloro-4-nitrophenol (p. 227, 358, 442)

4-Chloro-2-nitrophenol (p. 26, 76, 83, 155, 158, 182, 442)

4-Chloro-3-nitrophenol (p. 227)

5-Chloro-2-nitrophenol (p. 442)

4-Chloro-6-nitro-1-phenol-2-sulfonic acid (p. 90)

6-Chloro-2-nitro-1-phenol-4-sulfonic acid (p. 90)

O-2-Chloro-4-nitrophenyl-O,O-diethyl phosphorothioate
  American Cyanamid Linden, NJ

2-Chloro-5-nitrophenyl methyl sulfone
  Toms River Toms River, NJ

4-Chloro-3-nitrophenyl methyl sulfone
  Toms River Toms River, NJ

2-Chloro-3-nitropyridine
  Olin Rochester, NY

2-Chloro-5-nitropyridine
  Olin Rochester, NY

2'-Chloro-3-nitrosalicylanilide (p. 447)

2'-Chloro-5-nitrosalicylanilide (p. 447)

3'-Chloro-3-nitrosalicylanilide (p. 447)

3'-Chloro-5-nitrosalicylanilide (p. 447)

4'-Chloro-3-nitrosalicylanilide (p. 446, 447)

4'-Chloro-5-nitrosalicylanilide (p. 447)

α-Chloro-m-nitrotoluene
  Eastman Kodak Rochester, NY

2-Chloro-4-nitrotoluene (p. 6, 90, 368)
  Dupont Deepwater, NJ

2-Chloro-6-nitrotoluene (6-Chloro-2-nitrotoluene) (p. 6, 27, 76, 91, 360)
Dupont Deepwater, NJ

4-Chloro-2-nitrotoluene (p. 6, 27, 49, 60, 64, 76, 83)
American Color and Chem. Lock Haven, PA
Synalloy (Blackman Uhler) Spartanburg, SC

4-Chloro-3-nitrotoluene (p. 6, 49, 55, 91)
Synalloy (Blackman Uhler) Spartanburg, SC

1-Chloro-2,4,6-trinitrobenzene (2,4,6-Trinitrochlorobenzene; Picryl chloride) (p. 30, 97, 115, 398, 399)
Northrop Asheville, NC

2-Cyano-4-nitroanisole (2-Methoxy-5-nitrobenzonitrile) (p. 39)

DCNA (see 2,6-Dichloro-4-nitroaniline)

Diaminohexanitrobiphenyl
Northrop Asheville, NC

Diaminonitrotoluene (Nitrodiaminotoluene) (p. 177)

Diaminotrinitrobenzene (p. 91)
Northrop Asheville, NC

Diazodinitrophenol (2-Diazo-4,6-dinitrophenol) (p. 91)
Hercules Kenvil, NJ

2,6-Dibromo-4-nitroaniline (p. 91)
Martin Marietta Sodyeco, NC

2,6-Dibromo-4-nitrophenol (p. 236, 333, 334, 442)
Sherwin-Williams St. Bernard, OH

4,6-Dibromo-2-nitrophenol (p. 442)

2,6-Di-tert-butyl-4-nitrophenol (BNP) (p. 213, 214)

2,6-Dibutyl-4-nitrophenol (p. 359)

2',5-Dichloro-3-tert-butyl-4'-nitrosalicylanilide (p. 264, 265)

1,2-Dichloro-4,5-dinitrobenzene (p. 354)

2,5-Dichloro-4,6-dinitrophenol (p. 444)

2,5-Dichloro-4-nitroaniline (p. 19)

2,6-Dichloro-4-nitroaniline (DCNA; 1-Amino-2,6-dichloro-4-nitrobenzene) (p. 6, 19, 50, 53, 55, 60, 64, 74, 76, 84, 120, 236, 237, 264, 338, 348, 351, 376, 377, 388, 389, 415, 453, 493)
GAF Rensselaer, NY
Kewanee (Harshaw) Louisville, KY
Upjohn North Haven, CT

1,2-Dichloro-4-nitrobenzene (3,4-Dichloro-1-nitrobenzene) (p. 3, 7, 26, 59, 63, 82, 122, 193, 354)
Blue Spruce Edison, NJ

1,3-Dichloro-4-nitrobenzene (p. 44, 91, 122)
RSA Ardsley, NY

1,4-Dichloro-2-nitrobenzene (2,5-Dichloro-1-nitrobenzene) (p. 3, 7, 26, 48, 60, 64, 74, 83, 91, 122, 193, 354)
Dupont Deepwater, NJ
Mobay Bayonne, NJ

2,3-Dichloronitrobenzene (p. 122, 193)

2,4-Dichloro-1-nitrobenzene (see 1,3-Dichloro-4-nitrobenzene)

2,5-Dichloro-3-nitrobenzoic acid (p. 7, 91, 371)
GAF Linden, NJ

Dichloronitrobenzoic acid, isomeric mixture (p. 91)
GAF Linden, NJ

2,5-Dichloro-3-nitrobenzoic acid, ammonium salt
GAF Linden, NJ

2,5-Dichloro-3-nitrobenzoic acid, iminodi-2,2'-ethanol salt
GAF Linden, NJ

2,5-Dichloro-6-nitrobenzoic acid, sodium salt

1,2-Dichloro-3-nitronaphthalene (p. 429, 430, 432)

2,3-Dichloro-4-nitrophenol (p. 479, 480)

2,4-Dichloro-6-nitrophenol (4,6-Dichloro-2-nitrophenol) (p. 91, 442)

2,5-Dichloro-4-nitrophenol (p. 440, 442)

2,5-Dichloro-4-nitrophenol, sodium salt (p. 440)

2,6-Dichloro-4-nitrophenol (p. 158, 182, 358, 442)

4,5-Dichloro-2-nitrophenol (p. 442)

2',5'-Dichloro-3-nitrosalicylanilide (p. 448)

2',5-Dichloro-4'-nitrosalicylanilide (Niclosamide) (p. 91, 446)
Mobay Kansas City, MO

2',5-Dichloro-4'-nitrosalicylanilide, 2-aminoethanol salt
Mobay Kansas City, MO

2,4-Dichlorophenyl-4-nitrophenyl ether (2,4-Dichlorophenyl-*p*-nitrophenyl ether; Nitrofen) (p. 7, 42, 91, 347, 371)
Rohm and Haas Philadelphia, PA

N^3,N^3-Diethyl-2,4-dinitro-6-trifluoromethyl-*m*-phenylenediamine (Dinitramine) (p. 120, 451)

O,O-Diethyl-O-(*p*-nitrophenyl phosphorothioate) (Parathion, ethyl parathion) (p. 7, 49, 53, 57, 62, 77, 80, 81, 119, 120, 121, 247, 302)
Monsanto Anniston, AL
Stauffer Mt. Pleasant, TN

1,5-Difluoro-2,4-dinitrobenzene
Pierce Rockford, IL

2,4-Difluoronitrobenzene
Olin Rochester, NY

2,5-Difluoronitrobenzene
Olin Rochester, NY

4,5-Difluoro-2-nitrophenol (p. 442)

4,6-Difluoro-2-nitrophenol (p. 442)

2,6-Diiodo-4-nitrophenol (p. 91, 358, 359)

1,4-Dimethoxy-2-nitrobenzene (p. 55)

2,5-Dimethoxy-4'-nitrostilbene
Upjohn Kalamazoo, MI

O,O-Dimethyl-O-(3-methyl-4-nitrophenol) phosphorothioate (p. 42)

N,N-Dimethyl-*m*-nitroaniline (p. 92)

N,N-Dimethyl-*o*-nitroaniline (p. 92)

O,O-Dimethyl-O-(*p*-nitrophenyl)phosphorothioate (Methyl parathion) (p. 7, 49, 53, 57, 58, 62, 76, 80, 81, 120, 121)
Hercules Plaquemine, LA
Kerr-McGee Hamilton, MS
Monsanto Anniston, AL
Stauffer Mt. Pleasant, TN
Vicksburg Vicksburg, MS

2',3'-Dimethyl-3-nitrosalicylanilide (p. 448)

2',4'-Dimethyl-3-nitrosalicylanilide (p. 448)

2',6'-Dimethyl-3-nitrosalicylanilide (p. 448)

N,N-Dimethyl-3-nitro-*p*-toluenesulfonamide
GAF   Rensselaer, NY

2,4'-Dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene (p. 44)

2',4-Dimethyl-3,3',5,5'-tetranitro-ONN-azoxybenzene (p. 44)

2,4-Dinitroacetanilide (p. 55)

2,4-Dinitro-6-*sec*-amylphenol (p. 41)

2,4-Dinitroaniline (p. 7, 26, 50, 55, 60, 64, 74, 76, 82, 83, 92, 113, 351)
American Color and Chem.   Lock Haven, PA
Marin Marietta   Sodyeco, NC

o-(2,4-Dinitroanilino)phenol (p. 92)

p-(2,4-Dinitroanilino)phenol (p. 7, 49, 92)
GAF   Rensselaer, NY

2,4-Dinitroanisole (p. 8, 92, 333, 334, 371, 415)
Am. Hoechst   Somerville, NJ
Chemtronics   Swannanoa, NC

4,6-Dinitroanthranil (p. 44)

3,3'-Dinitroazoxybenzene (p. 224)

3,5-Dinitrobenzamide
Salsbury Labs   Charles City, LA

3',4-Dinitrobenzanilide (p. 8, 50, 92)
Toms River   Toms River, NJ

1,2-Dinitrobenzene (*o*-Dinitrobenzene) (p. 22, 122, 134, 136, 182, 191, 495, 496, 497)

1,3-Dinitrobenzene (*m*-Dinitrobenzene; 2,4-Dinitrobenzene; 2,6-Dinitrobenzene) (p. 8, 22, 27, 30, 33, 55, 59, 62, 76, 81, 114, 122, 124, 134, 136, 137, 138, 182, 191, 223, 224, 225, 245, 293, 334, 339, 340, 354, 377, 437, 466, 477, 481, 494, 495, 496, 497)
Dupont   Wilmington, DE

p-Dinitrobenzene (1,4-Dinitrobenzene) (p. 22, 134, 136, 137, 138, 182, 191, 354, 495, 496, 497)

2,4-Dinitrobenzenesulfonic acid (p. 92)
Eastman Kodak   Rochester, NY
Toms River   Toms River, NJ

2,4-Dinitrobenzenesulfonic acid, sodium salt (p. 92)
Frank   Columbus, OH
Hesse   State College, PA

3,5-Dinitrobenzenesulfonic acid (p. 128)

2,2'-Dinitrobenzidine (p. 92)

3,3'-Dinitrobenzidine (p. 92)
Northrop   Asheville, NC

2,4-Dinitrobenzoic acid (p. 142, 151, 182, 275)

2,5-Dinitrobenzoic acid (p. 142, 151, 182)

3,4-Dinitrobenzoic acid (p. 142, 151, 182)

3,5-Dinitrobenzoic acid (3,5-Dinitrobenzoate) (p. 55, 60, 64, 84, 151, 182)
Ashland   Great Meadows, NJ
Bofors   Linden, NJ
Salsbury Labs   Charles City, IA

3,5-Dinitrobenzoic acid, sodium salt (3,5-Dinitro-Na-benzoate) (p. 143, 150)

3,5-Dinitrobenzoyl chloride (p. 55, 92)
Bofors Linden, NJ
Eastman Kodak Rochester, NY
Guardian (Eastern) Hauppauge, NY

2,4'-Dinitrobiphenyl
Northrop Asheville, NC

4,4'-Dinitrobiphenyl (p. 8, 428)
Northrop Asheville, NC

Dinitrobutylphenol, ammonium salt (4,6-Dinitro-*o*-*sec*-butylphenol, ammonium salt) (p. 8, 52, 74, 92)
Dow Chem. Midland, MI

4,6-Dinitro-*o*-*sec*-butylphenol, triethanolamine salt (see 2-*sec*-Butyl-4,6-dinitrophenol, triethanolamine salt)

2,4-Dinitro-*o*-cresol (p. 93, 128, 158)

2,6-Dinitro-*p*-cresol (p. 329, 363)

4,6-Dinitro-*o*-cresol (DNOC; Dinitro-*o*-cresol; 2,4-Dinitro-6-methylphenol) (p. 8, 41, 55, 93, 129, 156, 162, 164, 165, 182, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 219, 220, 270, 272, 299, 300, 301, 304, 306, 308, 321, 323, 327, 328, 329, 331, 333, 334, 346, 349, 361, 362, 378, 385, 386, 406, 416, 443, 452, 454, 493, 496)
Blue Spruce Edison, NJ

4,6-Dinitro-*o*-cresol, sodium salt (p. 93, 301, 302, 303, 493)
Blue Spruce Edison, NJ

4,6-Dinitro-*o*-cyclohexyl phenol (2-Cyclohexyl-4,6-dinitrophenol) (p. 41, 93, 212, 213, 327, 329, 349, 350, 363, 376, 385, 495)

2,4-Dinitrodiazobenzene (p. 93)

3,4-Dinitro-dimethylaniline (isomer unknown) (p. 416)

4,4'-Dinitrodiphenylamine (p. 92, 93)

2,6-Dinitro-N,N-dipropyl cumidine (p. 371)

2,6-Dinitro-N,N-dipropyl-*p*-toluidine
Eli Lilly Lafayette, IN

2,5-Dinitrofluorene (p. 416, 428)

2,7-Dinitrofluorene (p. 411, 416, 428)

2,7-Dinitrofluoren-9-one
Mackenzie Chem. Works Central Islip, NY

2,4-Dinitro-5-fluoroaniline
Pierce Rockford, IL

Dinitrofluorobenzene

3',5'-Dinitro-2'-hydroxyacetanilide
Toms River Toms River, NJ

2,4-Dinitro-6-hydroxylaminotoluene (p. 177)

2,6-Dinitro-4-hydroxyalminotoluene (p. 177, 235)

1-(3,5-Dinitro-2-hydroxyphenylazo)-2-naphthol
Toms River Toms River, NJ

2,6-Dinitro-4-isopropylphenol (p. 365)

1,5-Dinitronaphthalene (p. 27, 497)

2,4-Dinitro-α-napthol (p. 8, 207, 208, 209, 210, 334, 372)
Carroll Wood River, RI

2,4-Dinitro-6-octylphenyl crotonate (Karathane; Dinocap; 2-Capryl-4,6-dinitrophenyl crotonate; Dinitrocapryl phenylcrotonate; 4,6-Dinitro-2-(1-methyl heptyl)phenyl crotonate) (p. 8, 92, 93, 94, 210, 211, 304, 347 350, 363, 372, 378, 452, 493)
Rohm & Haas Bristol, PA
Philadelphia, PA

2,4-Dinitrophenetole (p. 333, 334, 372)

2,3-Dinitrophenol (p. 329, 495)

2,4-Dinitrophenol (DNP) (p. 8, 26, 49, 53, 55, 59, 63, 76, 82, 83, 155, 156, 159, 162, 163, 164, 183, 191, 207, 208, 209, 210, 223, 224, 241, 242, 245, 259, 262, 263, 264, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 298, 299, 306, 307, 308, 323, 324, 325, 326, 327, 329, 331, 333, 334, 335, 336, 337, 338, 346, 349, 350, 363, 364, 365, 375, 378, 379, 385, 386, 387, 399, 403, 404, 405, 406, 407, 416, 444, 454, 457, 458, 459, 460, 467, 468, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 489, 490, 491, 492, 494, 495, 497)
Martin Marietta   Sodyeco, NC

2,5-Dinitrophenol (p. 155, 162, 163, 164, 183, 191, 259, 329, 471, 495)

2,6-Dinitrophenol (p. 55, 155, 162, 163, 164, 165, 183, 191, 329, 333, 334, 471, 495)

3,4-Dinitrophenol (p. 329, 495)

3,5-Dinitrophenol (p. 191, 329, 495)

2,6-Dinitro-1-phenol-4-sulfonic acid (p. 93)

2,4-Dinitrophenoxyethanol
Hummel   S. Plainfield, NJ

(2,4-Dinitrophenyl)hydrazine
Guardian   Hauppauge, NY
(Eastern)

2,4-Dinitro-6-phenylphenol (p. 365)

Dinitrophenyllysine hydrochloride (p. 426)

Dinitro-*o*-propylphenol (p. 386)

2,4-Dinitroresorcinol (p. 93, 157, 167, 183, 372)

3,5-Dinitrosalicylic acid
Eastman Kodak   Rochester, NY
Salsbury Labs   Charles City, IA

4,4'-Dinitrostilbene (p. 27)

4,4'-Dinitrostilbene-2,2'-disulfonic acid (p. 9, 27, 52, 55, 59, 62, 82)
American Cyanamid   Bound Brook, NJ
Ciba-Geigy   McIntosh, AL
GAF   Rensselaer, NY
Toms River   Toms River, NJ

2,4-Dinitrothymol (p. 334)

3,5-Dinitrotoluamide (Dinitolmide) (p. 9, 297, 372, 379, 399, 496)
Dow Chem.   Gainesville, GA
Midland, MI

2,3-Dinitrotoluene (p. 368, 496)

2,5-Dinitrotoluene (p. 19, 368, 496)

2,4-Dinitrotoluene (p. 9, 16, 19, 20, 29, 33, 36, 48, 55, 66, 68, 69, 76, 105, 113, 114, 128, 129, 169, 171, 172, 174, 183, 259, 316, 334, 340, 344, 368, 393, 394, 461, 462, 496)
Air Products and Chemicals   Pensacola, FL
Dupont   Deepwater, NJ
Rubicon   Geismar, LA

2,4-(and 2,6)-Dinitrotoluenes (p. 9, 16, 20, 48, 53, 58, 61, 66, 80, 87, 103, 104, 107, 108, 109, 128, 129, 174, 369, 379, 496)
Dupont   Deepwater, NJ
Mobay   Cedar Bayou, TX
New Martinsville, WV

2,6-Dinitrotoluene (p. 341, 369, 496)

3,4-Dinitrotoluene (p. 129, 369, 496)

3,5-Dinitrotoluene (p. 19, 496)

Dinitrotoluene Oil (Dinitrotoluenes) (p. 16, 20, 280, 496)

2,4-Dinitrotoluene-3-sulfonic acid (p. 128)

2,4-Dinitrotoluene-5-sulfonic acid (p. 128)

3,5-Dinitro-*p*-toluenesulfonic acid
GAF   Rensselaer, NY

3,5-Dinitro-*p*-toluidine (p. 372)

Dinitrotrichlorobenzene (p. 355)

2,4'-Dinitro-4-trifluoromethyldiphenyl ether (p. 55, 93)
Ciba-Geigy   McIntosh, AL

2,4-Dinitro-1,3,5-trimethylbenzene (2,4-Dinitromesitylene) (p. 334, 342)

4,6-Dinitro-1,3-xylene (p. 341)

Dinocap (see 2,4-Dinitro-6-octylphenyl crotonate)

Dinoseb (see 2-*sec*-Butyl-4,6-dinitrophenol)

5,5-Dithiobis-(2-nitrobenzoic acid)
Pierce   Rockford, IL

DNCB (see 1-Chloro-2,4-dinitrobenzene)

DNFB (see 1-Fluoro-2,4-dinitrobenzene)

DNOC (see 4,6-Dinitro-*o*-cresol)

DNP (see 2,4-Dinitrophenol)

4-Ethoxy-3-nitroacetanilide
American Color and Chem.   Lock Haven, PA

4-Ethyl-2,6-dinitrophenol (p. 365)

N,N'-Ethylenebis(3-nitrobenzenesulfonamide)
Salsbury Labs   Charles City, IA

Ethyl-*m*-nitrobenzoate (p. 38)

Ethyl-*p*-nitrobenzoate (p. 33, 38)

1-Fluoro-2,4-dinitrobenzene (DNFB; 2,4-Dinitrofluorobenzene) (p. 9, 219, 260, 355, 399, 424, 425, 426)
Eastman Kodak   Rochester, NY
Olin   Rochester, NY
Pierce Chem.   Rockford, IL

2-Fluoro-3,5-dinitro-benzotrifluoride (p. 89)

4-Fluoro-2,6-dinitrophenol (p. 444)

2-Fluoro-5-nitroaniline
Olin   Rochester, NY

4-Fluoro-2-nitroaniline
Olin   Rochester, NY

4-Fluoro-3-nitroaniline
Olin   Rochester, NY

m-Fluoronitrobenzene (p. 122)
Olin   Rochester, NY

o-Fluoronitrobenzene (p. 122)
Olin   Rochester, NY

p-Fluoronitrobenzene (p. 9, 122, 355)
Olin   Rochester, NY

2-Fluoro-4-nitrobenzoic acid (2-Fluoro-4-nitrobenzoate) (p. 151, 152, 182)

3-Fluoro-4-nitrobenzoic acid (3-Fluoro-4-nitrobenzoate) (p. 142, 143, 182)

4-Fluoro-3-nitrobenzoic acid
Olin   Rochester, NY

2-Fluoro-4-nitrophenol (p. 442)

3-Fluoro-2-nitrophenol (p. 442)

3-Fluoro-4-nitrophenol (p. 442)

4-Fluoro-2-nitrophenol (p. 442)

5-Fluoro-2-nitrophenol (p. 442)

6-Fluoro-2-nitrophenol (p. 442)

2'-Fluoro-3-nitrosalicylanilide (p. 447)

3'-Fluoro-3-nitrosalicylanilide (p. 447)

4'-Fluoro-3-nitrosalicylanilide (p. 447)

4'-Fluoro-5-nitrosalicylanilide (p. 447)

2-Fluoro-4-nitrotoluene
Olin   Rochester, NY

2-Fluoro-5-nitrotoluene
Olin   Rochester, NY

4-Fluoro-2-nitrotoluene
Olin   Rochester, NY

Fluoro-2,4,6-trinitrobenzene (2,4,6-Trinitrofluorobenzene) (p. 30)

L-δ-Glutamyl-*p*-nitroanilide
Beckman   Carlsbad, CA

1,2,3,4,5,6-Hexachloro-7-nitronaphthalene (p. 416)

Hexanitroazobenzene
Northrop   Asheville, NC

Hexanitrodiphenylamine (p. 93, 434, 435)

Hexanitrodiphenyl sulfone
Northrop   Asheville, NC

Hexanitrostilbene (2,2',4,4',6,6'-Hexanitrostilbene) (p. 9, 416)
Northrop   Asheville, NC

2-Hydrazino-4-(*p*-nitrophenyl)thiazole (p. 417)

4-Hydroxylamino-2-nitrophenol (p. 163)

6'-Hydroxy-5'-[(2-hydroxy-5-nitrophenyl)azo]-*m*-acetotoluidide
Toms River   Toms River, NJ

N-[7-Hydroxy-8-([2-hydroxy-5-nitrophenyl]azo)-1-naphthyl]acetamide
Toms River   Toms River, NJ

4-Hydroxy-7(*p*-nitrobenzamido)-2-naphthalene sulfonic acid
GAF   Rensselaer, NY

4-Hydroxy-3-nitrobenzenearsonic acid
Salsbury Labs   Charles City, IA

4-Hydroxy-3-nitrobenzenearsonic acid, monosodium salt
Salsbury Labs   Charles City, IA

4-Hydroxy-3-nitrobenzenesulfonic acid (p. 93)

2-Hydroxy-4-nitrobenzoic acid (p. 182)

3-Hydroxy-4-nitrobenzoic acid (p. 142, 182)

2-Hydroxy-5-nitrometanilic acid
Toms River   Toms River, NJ

3-Hydroxy-3'-nitro-2-naphthanilide (p. 93)
Pfister   Ridgefield, NJ

1-(2-Hydroxy-4-nitrophenylazo)-2-naphthol
Toms River   Toms River, NJ

N-(2-Hydroxy-5-nitrophenyl)glycerine (p. 93)

2-Iodo-3-nitrobenzoic acid (p. 93)

2-Iodo-3-nitrophenol (p. 442)

2'-Iodo-3-nitrosalicylanilide (p. 447)

3'-Iodo-3-nitrosalicylanilide (p. 447)

4'-Iodo-3-nitrosalicylanilide (p. 447)

4'-Iodo-5-nitrosalicylanilide (p. 447)

2-(*p*-Iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazoliam chloride
Aldrich   Milwaukee, WI

Isopropyl-*p*-nitrobenzoate (p. 33)

2-Iodo-4-nitrobenzoic acid (2-Iodo-4-nitrobenzoate) (p. 142, 152, 182)

Karathane (see 2,4-Dinitro-6-octylphenyl crotonate)

Lead 2,4-dinitroresorcinate (p. 93)

Lead nitroresorcinol, mono
Tyler   Tamaqua, PA

2'-Methoxy-5'-chloro-3-nitrosalicylanilide (p. 448)

6-Methoxy-8-nitroquinoline
Sterling Drug   Rensselaer, NY

2-(α-Methylbenzyl)-4,6-dinitrophenol (p. 365)

2'-Methyl-3'-chloro-3-nitrosalicylanilide (p. 448)

2'-Methyl-5'-chloro-3-nitrosalicylanilide (p. 448)

1-Methyl-3,4-dimethoxynitrobenzene
Orbis Products   Newark, NJ

4,4'-Methylenebis(N,N-dimethyl-3-nitroaniline)
GAF   Rensselaer, NY

N-Methyl-4'-nitroacetanilide
GAF   Linden, NJ

N-Methyl-*p*-nitroaniline
GAF   Linden, NJ

2-Methyl-5-nitroaniline
Pfister   Ridgefield, NJ

3-Methyl-2-nitrobenzoic acid (2-Nitro-*m*-toluic acid) (p. 56, 182)
Salsbury Labs   Charles City, IA

3-Methyl-4-nitrobenzoic acid
Bofors   Linden, NJ
Salsbury Labs   Charles City, IA

3-Methyl-6-nitrobenzoic acid
Salsbury Labs   Charles City, IA

2-Methyl-4-nitrophenol (p. 155, 183)

N-Methyl-N-nitro-2,4,6-trinitroaniline (p. 14, 29, 96, 110, 113, 114, 115, 118, 130, 291, 292, 314, 315, 352, 383, 397, 398, 417, 496)
Hummel   S. Plainfield, NJ

4-(Methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline (p. 9, 352)
Shell   Denver, CO

2-(Methylsulfonyl)-4-nitroaniline (p. 55)
Toms River   Toms River, NJ

Musk ambrette (see 6-*tert*-Butyl-3-methyl-2,4-dinitroanisole)

Nigrosine (mixture containing nitrobenzene, nitrophenol, or nitrocresols) (p. 297, 399)

3'-Nitroacetanilide (p. 50, 94)
GAF   Rensselaer, NY
Toms River   Toms River, NJ

4'-Nitroacetanilide (p. 50)
GAF   Rensselaer, NY
Toms River   Toms River, NJ

3'-Nitroacetanilide (p. 10)
GAF   Rensselaer, NY

3'-Nitroacetophenone (*m*-Nitroacetophenone) (p. 10, 245, 372)
Syntex   Newport, TN

4'-Nitroacetophenone (*p*-Nitroacetophenone) (p. 253)

3-Nitro-4-aminoanisole (see 2-Nitro-*p*-anisidine)

m-Nitroaniline (3-Nitroaniline) (p. 10, 27, 50, 55, 76, 122, 179, 180, 181, 182, 185, 191, 197, 223, 224, 225, 280, 352, 401, 484)

o-Nitroaniline (2-Nitroaniline; 2-Nitrophenylamine) (p. 10, 55, 59, 63, 77, 80, 82, 95, 122, 179, 180, 181, 182, 185, 191, 353, 484, 495)
Monsanto   Sauget, IL

p-Nitroaniline (4-Nitroaniline) (p. 10, 26, 33, 35, 38, 50, 53, 55, 58, 62, 74, 77, 78, 80, 81, 98, 104, 106, 107, 136, 178, 179, 180, 181, 182, 185, 191, 197, 217, 218, 236, 238, 239, 280, 305, 311, 312, 353, 379, 401, 484, 495, 496)
American Color and Chem.   Lock Haven, PA
Monsanto   Sauget, IL
Universal Oil Products   McCook, IL

4-Nitroaniline-3-sulfonic acid (5-Amino-2-nitrobenzenesulfonic acid; 6-Nitrometanilic acid) (p. 94)

2-Nitro-*p*-anisidine (4-Amino-3-nitroanisole; 3-Nitro-4-aminosole) (p. 19, 20, 55)
Dupont   Deepwater, NJ

4-Nitro-*o*-anisidine (2-Amino-5-nitroanisole; 4-Nitro-2-anisidine) (p. 10, 19, 49, 55, 74, 94)
Dupont   Deepwater, NJ

4-Nitro-3-anisidine (p. 94)

5-Nitro-*o*-anisidine (2-Amino-4-nitroanisole) (p. 10, 19, 49, 53, 55, 94, 372)
American Cyanamid   Marietta, OH
Synalloy (Blackman Uhler)   Spartanburg, SC

o-Nitroanisole (2-Nitroanisole) (p. 11, 20, 26, 55, 59, 63, 77, 83, 372)
Dupont   Deepwater, NJ
Monsanto   St. Louis, MO

p-Nitroanisole (4-Nitroanisole) (p. 11, 20, 33, 38, 39, 59, 63, 77, 83, 372, 379)
Dupont   Deepwater, NJ

m-Nitrobenzaldehyde (p. 28, 55, 245, 401)
Aldrich   Milwaukee, WI

o-Nitrobenzaldehyde (p. 28, 31, 94)
Aldrich   Milwaukee, WI
RSA   Ardsley, NY

p-Nitrobenzaldehyde (p. 11, 234, 245, 343, 373, 401)
Sterling Drug   Rensselaer, NY

o-Nitrobenzamide (p. 373)

4'-Nitrobenzanilide
GAF   Rensselaer, NY

Nitrobenzene (Mononitrobenzene) (p. 3, 11, 20, 21, 22, 23, 24, 27, 33, 34, 35, 37, 38, 40, 48, 53, 58, 61, 65, 66, 67, 74, 77, 78, 79, 80, 85, 86, 87, 104, 105, 106, 107, 123, 124, 125, 128, 129, 134, 136, 138, 182, 185, 188, 191, 192, 193, 214, 215, 216, 218, 221, 222, 223, 245, 246, 253, 254, 255, 278, 280, 282, 294, 309, 310, 311, 318, 319, 320, 339, 341, 343, 344, 345, 355, 356, 380, 390, 391, 392, 393, 399, 401, 411, 435, 436, 454, 495, 496)
Allied Chem.   Moundsville, WV
American Cyanamid   Bound Brook, NJ
Willow Island, W

Nitrobenzene (Cont'd)
Dupont Beaumont, TX
Gibbstown, NJ
First Mississippi Pascagoula, MS
Mobay New Martinsville, WV
Monsanto Sauget, IL
Rubicon Chem. Geismar, LA

p-Nitrobenzenesulfonamide (p. 249, 250, 373)

3'-Nitrobenzenesulfonanilide
GAF Rensselaer, NY

m-Nitrobenzenesulfonic acid (p. 51, 59, 63, 74, 82)
Toms River Toms River, NJ

m-Nitrobenzenesulfonic acid, potassium salt (p. 63)
American Cyanamid Bound Brook, NJ

m-Nitrobenzenesulfonic acid, sodium salt (p. 51, 55, 59, 63, 74, 82)
GAF Linden, NJ
USM (Crown Metro) Greenville, SC

m-Nitrobenzenesulfonyl chloride (p. 52)
GAF Rensselaer, NY

o-Nitrobenzenesulfonyl chloride (p. 94)

p-Nitrobenzenesulfonyl chloride
Eastman Kodak Rochester, NY

2-Nitrobenzidine (p. 94)

3-Nitrobenzidine (p. 94)

6-Nitrobenzimidazole
Fairmount Newark, NJ

6-Nitrobenzimidazole nitrate
Fairmount Newark, NJ

6-Nitrobenzimidazole, sodium salt
Fairmount Newark, NJ

m-Nitrobenzoic acid (m-Nitrobenzoate) (p. 11, 28, 38, 51, 55, 60, 63, 83, 141, 142, 143, 144, 145, 146, 147, 148, 150, 182, 191, 196, 245, 373)
Bofors Linden, NJ
Salsbury Labs Charles City, IA
Sterling Drug Cincinnati, OH

o-Nitrobenzoic acid (o-Nitrobenzoate) (p. 11, 31, 55, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 182, 191, 196, 231, 373, 472, 473, 474, 475)
Bofors Linden, NJ
Salsbury Labs Charles City, IA

p-Nitrobenzoic acid (PNBA; p-Nitrobenzoate; 4-Nitrobenzoate) (p. 11, 20, 27, 33, 38, 55, 77, 95, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 158, 182, 191, 231, 234, 245, 246, 249, 250, 251, 275, 373, 472)
Bofors Linden, NJ
Dupont Deepwater, NJ

m- and p-Nitrobenzoic acids (p. 11, 17, 51)

p-Nitrobenzoic acid, ethyl ester
Bofors Linden, NJ

m-Nitrobenzoic acid, sodium salt (Sodium-m-nitrobenzoate) (p. 11, 51, 60, 63, 83, 143, 145)
Salsbury Labs Charles City, IA

o-Nitrobenzoic acid, sodium salt (o-Nitro-Na-benzoate) (p. 145)

p-Nitrobenzoic acid, sodium salt (p-Nitro-Na-benzoate) (p. 143, 145)

m-Nitrobenzonitrile (p. 38)

p-Nitrobenzonitrile (p. 33, 37, 38, 39)

m-Nitrobenzotrifluoride
Olin Rochester, NY

6-Nitro-2-benzoxazolinone
GAF Rensselaer, NY

2-(*m*-Nitrobenzoyl)-*o*-acetanisidide
GAF Linden, NJ

m-Nitrobenzoyl chloride (p. 55)
Aceto Carlstadt, NJ
Bofors Linden, NJ

o-Nitrobenzoyl chloride
Bofors Linden, NJ

p-Nitrobenzoyl chloride (p. 55)
Occidental Petroleum (Hooker) Niagara Falls, NY

o-Nitrobenzyl alcohol (p. 231)

p-Nitrobenzyl alcohol (p. 234, 245)
Eastman Kodak Rochester, NY

p-Nitrobenzyl bromide (p. 95)
RSA Ardsley, NY
Stauffer Edison, NJ

4-(*p*-Nitrobenzyl)pyridine
Eastman Kodak Rochester, NY

o-Nitrobiphenyl (2-Nitrobiphenyl) (p. 12, 373, 381)

p-Nitrobiphenyl (4-Nitrobiphenyl) (p. 246, 373, 382, 411, 417, 427, 429, 496)

4'-Nitro-4-biphenylcarboxylic acid
Toms River Toms River, NJ

4-Nitrocatechol (p. 161, 221, 222)

3-Nitro-4-chloroacetanilide
Frank Enterprises Columbus, OH

2-Nitro-4-chloroaniline (see 4-Chloro-2-nitroaniline)

3-Nitro-4-chloroaniline (see 4-Chloro-3-nitroaniline)

2-Nitro-5-chloroanisole (see 5-Chloro-2-nitroanisole)

p-Nitrocresol (p. 246)

2-Nitro-*p*-cresol (p. 12, 77, 129, 366)
Sherwin-Williams Chicago, IL

4-Nitro-*m*-cresol (p. 56, 366)

6-Nitro-*m*-cresol

4-Nitro-6-cyclohexylphenol (p. 387)

2-Nitro-*p*-cymene
Eastman Kodak Rochester, NY

5-Nitro-1-diazo-2-naphthol-4-sulfonic acid (p. 55)

6-Nitro-1-diazo-2-naphthol-4-sulfonic acid (p. 95)

4-Nitro-N,N-diethylaniline (p. 35)

2-Nitrodiphenylamine
American Cyanamid Marietta, OH

4-Nitrodiphenylamine (p. 56)
Monsanto Sauget, IL

2-Nitrodiphenylamine-4-sulfonanilide
Salsbury Labs Charles City, IA

4-Nitrodiphenylamine-2-sulfonic acid (p. 95)

Nitrododecylbenzene
Monsanto Sauget, IL

4-Nitro-N-ethylaniline (p. 35)

2-Nitrofluorene (p. 246, 411, 417, 428, 430)

6-Nitro-2-furaldehyde semicarbazone (p. 417)

5-Nitrofuran (p. 411)

N-[4-(5-Nitro-2-furyl)-2-thiazolyl]-acetamide (p. 418) p-Nitrohippuric acid (p. 231)

Nitrohydroquinone (p. 161)

5-Nitroimidazole (p. 411)

5-Nitroisophthalic acid
  Ashland   Great Meadows, NJ
  Bofors   Linden, NJ
  GAF   Linden, NJ

1-Nitronaphthalene (α-Nitronaphthalene) (p. 12. 27, 59, 63, 82, 246, 373)
  Dupont   Wilmington, DE

2-Nitronaphthalene (β-Nitronaphthalene) (p. 246, 256, 374, 411, 418, 429)

3-Nitro-1,5-naphthalenedisulfonic acid (Nitro casella acid) (p. 12, 52)
  GAF   Linden, NJ
  Toms River   Toms River, NJ

1-Nitro-3,6,8-naphthalene trisulfonic acid (p. 27)

4-Nitronaphthalic anhydride
  GAF   Linden, NJ

7-(and 8-)Nitronapth(1,2-d)(1,2,3)oxadiazole-5-sulfonic acid (p. 12, 52, 60, 64, 84)
  GAF   Rensselaer, NY
  Mobay   Bayonne, NJ
      Charleston, SC
  Toms River   Toms River, NJ

3-Nitro-2-naphthylamine (p. 429, 430, 431)

p-Nitroperbenzoic acid (p. 418, 433)

3-Nitro-p-phenetidine (3-Nitro-p-ethoxyaniline) (p. 56)

4-Nitro-o-phenetidine (4-Nitro-o-ethoxyaniline) (p. 56)

5-Nitro-o-phenetidine (5-Nitro-o-ethoxyaniline) (p. 56)

5-Nitro-p-phenetidine (5-Nitro-p-ethoxyaniline) (p. 56)

m-Nitrophenol (3-Nitrophenol) (p. 56, 73, 95, 154, 155, 156, 158, 159, 160, 161, 183, 191, 221, 222, 223, 240, 245, 259, 275, 277, 366, 386, 387, 401, 444, 471, 497)

o-Nitrophenol (2-Nitrophenol) (p. 12, 35, 56, 58, 62, 73, 77, 81, 104, 129, 154, 155, 156, 158, 159, 160, 161, 183, 185, 189, 191, 193, 221, 222, 240, 258, 259, 275, 276, 366, 386, 387, 418, 437, 438, 439, 444, 457, 458, 459, 497)
  Martin Marietta   Sodyeco, NC
  Monsanto   Sauget, IL

p-Nitrophenol (4-Nitrophenol) (p. 12, 20, 26, 33, 35, 38, 49, 53, 56, 57, 58, 61, 73, 74, 78, 79, 80, 101, 112, 119, 120, 125, 129, 130, 154, 155, 156, 158, 159, 160, 161, 183, 191, 192, 193, 207, 208, 209, 210, 215, 216, 221, 222, 223, 240, 245, 274, 275, 277, 313, 331, 366, 367, 382, 386, 387, 401, 418, 443, 444, 457, 458, 459, 471, 480, 482, 494, 497)
  Dupont   Deepwater, NJ
  Martin Marietta   Sodyeco, NC
  Monsanto   Anniston, AL
      Sauget, IL
  Northern Fine   Franklin, NJ
  G.D. Searle (Will Ross)   Norwood, OH

p-Nitrophenol, sodium salt (sodium p-Nitrophenate) (p. 20, 49, 53, 58, 61, 80)
  Dupont   Deepwater, NJ
  Northern Fine   Franklin, NJ

6-Nitro-1-phenol-2,4-disulfonic acid (p. 95)

p-Nitrophenyl acetate
Eastman Kodak Rochester, NY

(p-Nitrophenyl)acetic acid (p-Nitro-α-toluic acid)
Stauffer Chem. Edison, NJ

4'-(p-Nitrophenyl) acetophenone (p. 12, 52, 95)
GAF Linden, NJ

2-Nitrophenylamine (see o-Nitroaniline)

4-Nitrophenylarsonic acid (p. 95)
Salsbury Charles City, IA

2-(o-Nitrophenylazo)-p-cresol
Toms River Toms River, NJ

2-Nitro-p-phenylenediamine (2-NPPD; m-Nitro-p-phenylenediamine) (p. 12, 18, 56, 95, 401, 402, 403, 433)
Ashland Great Meadows, NJ
Martin Marietta Sodyeco, NC
Olin Rochester, NY

4-Nitro-o-phenylenediamine (4-NOPD; Nitro-o-phenylenediamine) (p. 13, 18, 56, 94, 95, 401, 402, 433)
Ashland Great Meadows, NJ
Fairmount Chem. Newark, NJ

4-Nitro-m-phenylenediamine (p. 56, 95)

5-Nitro-m-phenylenediamine (p. 95)

p-Nitrophenyl ethyl ether (p. 26)

N-(p-Nitrophenyl) glycine (p. 95)

(p-Nitrophenyl)hydrazine
Eastman Kodak Rochester, NY

m-Nitrophenylhydroxylamine (p. 95)

p-Nitrophenyl isocyanate
Eastman Kodak Rochester, NY

p-Nitrophenylmercapturic acid (p. 221)

2-(4-Nitrophenyl)-(2H)-naphtho(1,2-d)-triazole-6,8-disulfonic acid
Toms River Toms River, NJ

2-(p-Nitrophenyl)-1-octadecyl-5-benzimidazolesulfonic acid
GAF Linden, NJ

1-(m-Nitrophenyl)-5-oxo-2-pyrazoline-3-carboxylic acid (p. 56)
Mobay Bayonne, NJ

p-Nitrophenylphosphate, disodium salt (p. 56)
Aldrich Milwaukee, WI

p-Nitrophenyl phosphate, sodium salt
Regis Morton Grove, IL

3-(2-Nitrophenyl)propenoic acid (p. 28, 31)

o-Nitrophenylsulfenyl chloride
Pierce Rockford, IL

Bis(4-Nitrophenyl)sulfide
American Cyanamid Willow Island, WV

p-Nitrophenyl-α,α,α-trifluoro-2-nitro-p-tolyl ether
Nor-Am Chicago, IL

3-Nitrophthalic acid
Eastman Kodak Rochester, NY

3-Nitrophthalic anhydride
Eastman Kodak Rochester, NY

4-Nitrophthalimide
Martin Marietta Sodyeco, NC

p-Nitrophenylphosphate
Pierce Rockford, IL

4-Nitropyridine-N-oxide
Aldrich Milwaukee, WI

4-Nitropyrogallol (p. 96)

Nitroquinol (p. 221)

4-Nitroquinoline-N-oxide (p. 411, 414, 419)

5-Nitrosalicylaldehyde
Eastman Kodak Rochester, NY

Nitrosalicylanilides (p. 406, 446)

3-Nitrosalicylanilide (p. 446)

3-(and 5-)Nitrosalicylic (p. 195, 196)
GAF Rensselaer, NY

p-Nitrosodium phenolate (see p-Nitrophenol, sodium salt)

4-Nitroso-2-nitrophenol (p. 163)

4-Nitrostilbene (p. 13, 411, 418)
GAF Rensselaer, NY

4-Nitro-4'-(5-sulfo-2H-naphthol(1,2d)-triazol-2-yl)-2,2'-stilbenedisulfonic acid
Toms River Toms River, NJ

4-Nitro-2-sulfotoluene (see 5-Nitro-o-toluenesulfonic acid)

m-Nitrotoluene (3-Nitrotoluene) (p. 13, 16, 33, 77, 96, 129, 185, 191, 193, 277, 342, 369, 393, 394, 399, 454, 496)
First Mississippi Pascagoula, MS

o-Nitrotoluene (2-Nitrotoluene) (p. 13, 16, 20, 24, 27, 31, 33, 36, 59, 62, 66, 69, 77, 81, 125, 129, 185, 191, 193, 231, 277, 280, 341, 370, 393, 394, 399, 454, 461, 462, 496)
Dupont Deepwater, NJ
First Mississippi Pascagoula, MS

p-Nitrotoluene (4-Nitrotoluene) (p. 13, 16, 20, 27, 33, 38, 48, 56, 58, 62, 66, 69, 77, 81, 118, 125, 129, 169, 171, 183, 185, 189, 191, 231, 234, 245, 253, 277, 280, 339, 342, 343, 370, 393, 394, 399, 401, 454, 496)

p-Nitrotoluene (Cont'd)
Dupont Deepwater, NJ
First Mississippi Pascagoula, MS

Nitrotoluenes, mixed (p. 280, 496)
Dupont Deepwater, NJ

5-Nitro-o-toluenesulfonanilide
GAF Linden, NJ

3-Nitro-p-toluenesulfonic acid (p. 13, 51, 96)
Nyanza Ashland, MA
Toms River Toms River, NJ

4-Nitrotoluene-2-sulfonic acid (4-Nitro-o-toluenesulfonic acid) (p. 13)
Dupont Deepwater, NJ

5-Nitro-o-toluenesulfonic acid (4-Nitro-2-sulfotoluene) (p. 52, 59, 62, 81, 82, 96)
American Cyanamid Bound Brook, NJ
Dupont Deepwater, NJ
GAF Rensselaer, NY
Toms River Toms River, NJ

4'-Nitro-p-toluenesulfono-o-toluidide
GAF Rensselaer, NY

5-Nitro-o-toluenesulfonyl chloride
GAF Linden, NJ

3-Nitro-o-toluic acid (2-Methyl-3-nitrobenzoic acid) (p. 56)

3-Nitro-p-toluic acid (4-Methyl-3-nitrobenzoic acid) (p. 56)

2-Nitro-p-toluidine (4-Amino-3-nitrotoluene) (p. 27, 50, 53, 56, 60, 63, 74, 77, 83)
Sherwin-Williams Chicago, IL

3-Nitro-p-toluidine (4-Amino-2-nitrotoluene) (p. 13, 28, 374, 461, 462)

4-Nitro-o-toluidine (2-Amino-5-nitrotoluene) (p. 13, 50, 56, 74)
GAF Rensselaer, NY

5-Nitro-o-toluidine (2-Amino-4-nitrotoluene) (p. 14, 50, 53, 56, 368, 374)
  Dupont   Deepwater, NJ
  Pfister Chem.   Ridgefield, NJ
  Synalloy   Spartanburg, SC
  (Blackman Uhler)

5-Nitro-2-p-toluidinobenzenesulfonic acid
  Toms River   Toms River, NJ

2-Nitro-4-trifluoromethylchlorobenzene
  Pfister Chem.   Ridgefield, NJ

7-Nitro-2-trifluoromethylphenothiazine
  Olin   Rochester, NY

3-Nitro-α,α,α-trifluorotoluene (α,α,α-Trifluoro-m-nitrotoluene) (p. 370)
  Olin   Rochester, NY

p-Nitro-o-xylene (p. 56)

Nitroxylenes (p. 14, 48)

4-Nitro-2,6-xylenol (p. 374)

4-NOPD (see 4-Nitro-o-phenylenediamine)

2-NPPD (see 2-Nitro-p-phenylenediamine)

Parathion (see O,O-Diethyl-O-[p-nitrophenyl]-phosphorothioate)

Pentachloronitrobenzene (PCNB) (p. 3, 14, 42, 56, 96, 119, 120, 186, 187, 190, 192, 193, 230, 231, 232, 233, 297, 345, 348, 357, 382, 399, 401, 410, 419, 420, 421, 422, 423, 469, 476, 493)
  Olin   McIntosh, AL

1,1,3,3,5-Pentamethyl-4,6-dinitroindan (p. 96)
  Givaudan   Clifton, NJ

Picramic acid (see 2-Amino-4,6-dinitrophenol)

Picramic acid, sodium salt (see 2-Amino-4,6-dinitrophenol, sodium salt)

Picramide (p. 434, 435)

Picric acid (see 2,4,6-Trinitrophenol)

Picric acid, ammonium salt (see 2,4,6-Trinitrophenol, ammonium salt)

Picric acid, sodium salt (see 2,4,6-Trinitrophenol, sodium salt)

Picrolonic acid (3-Methyl-4-nitro-1-(p-nitrophenyl)-5-pyrazolone)
  RSA   Aresley, NY

Picryl chloride (see 1-Chloro-2,4,6-trinitrobenzene)

PNBA (see p-Nitrobenzoic acid)

Sodium-m-nitrobenzenesulfonate
  GAF   Linden, NJ
  Monsanto   St. Louis, MO
  Salsbury Labs   Charles City, IA

Sodium picramate (see 2-Amino-4,6-dinitrophenol, sodium salt)

Tetrachloronitroanisole (p. 119)

Tetrachloronitrobenzene (1,2,4,5-Tetrachloro-3-nitrobenzene) (p. 3, 14, 96, 119, 120)
  Aceto   Flushing, NY

2,3,4,5-Tetrachloronitrobenzene (p. 193, 228, 229, 230, 247, 419, 420, 421)

2,3,4,6-Tetrachloronitrobenzene (p. 193, 419, 420, 421)

2,3,5,6-Tetrachloronitrobenzene (p. 193, 228, 229, 247, 419, 420, 421, 422, 493)
  Sterling   Cincinnati, OH

2,3,5,6-Tetrachloro-4-nitrophenol (p. 442)

2,3,4,6-Tetranitroaniline (p. 14, 345, 353)
Hummel Chem. S. Plainfield, NJ

2,2',6,6'-Tetranitro-4,4'-azoxytoluene (p. 44, 177, 235)

4,4',6,6'-Tetranitro-2,2'-azoxytoluene (p. 44, 177)

1,2,4,6-Tetranitrobenzene (p. 30)

Tetranitrofluoren-9-one
Mackenzie Central Islip, NY

Tetranitroxylene (p. 374)

Tetryl (see N-Methyl-N-nitro-2,4,5-trinitroaniline)

2,2'-Thiobis(5-nitrobenzenesulfonic acid)
GAF Rensselaer, NY

D-Threo-1-(p-nitrophenyl)-2-amino-1,3-propanediol
Warner-Lambert Holland, MI

Triaminotrinitrobenzene
Northrop Asheville, NC

2,3,5-Tri-O-benzyl-1-O-p-nitrobenzoyl-D-arabinofuranose
Pfanstiehl Waukegan, IL

1,2,4-Trichloro-5-nitrobenzene (p. 15, 357)
Alliance Newark, NJ
Pfister Ridgefield, NJ

2,3,4-Trichloronitrobenzene (p. 193)

2,4,5-Trichloronitrobenzene (p. 122, 193)

2,3,6-Trichloro-4-nitrophenol (p. 442)

3,4,6-Trichloro-2-nitrophenol (p. 440, 442, 479, 480)

3,4,6-Trichloro-2-nitrophenol, sodium salt (p. 440)

α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine (Trifluralin) (p. 15, 34, 42, 58, 62, 81, 247, 248, 348, 372, 449, 450, 452, 493)
Eli Lilly Lafayette, IN

2-Trifluoromethyl-4-nitrophenol (p. 442, 443)

3-Trifluoromethyl-2-nitrophenol (p. 442)

3-Trifluoromethyl-4-nitrophenol (p. 96, 120, 367, 440, 441, 442, 443, 445, 446, 451)

3-Trifluoromethyl-4-nitrophenol, sodium salt (p. 440)

4-Trifluoromethyl-2-nitrophenol (p. 442)

Trifluralin (see α,α,α-Trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine)

2,4,6-Trinitroaniline (p. 96)

2,4,6-Trinitroanisole (p. 30, 374, 396)

2,4,6-Trinitrobenzaldehyde (p. 44)

1,2,3-Trinitrobenzene (p. 22, 495)

1,3,5-Trinitrobenzene (2,4,5-Trinitrobenzene) (p. 22, 29, 44, 45, 96, 115, 124, 134, 136, 137, 138, 182, 339, 340, 383, 495)

2,4,6-Trinitrobenzenesulfonic acid (p. 15)
Pierce Rockford, IL

2,4,6-Trinitrobenzoic acid (p. 96, 129, 142, 151, 182, 275, 495)

2,4,6-Trinitrobenzoic acid, sodium salt (p. 143, 150)

2,4,6-Trinitrobenzonitrile (p. 44)

2,4,6-Trinitrobenzyl alcohol (p. 235)

2,4,6-Trinitro-m-cresol (p. 156, 165, 182, 329, 367)

2,4,7-Trinitrofluoren-9-one
Eastman Kodak Rochester, NY
MacKenzie Chem. Central Islip, NY

2,4,6-Trinitrophenetole (p. 398)

2,4,6-Trinitrophenol (Picric acid) (p. 14, 29, 77, 82, 97, 113, 155, 156, 162, 163, 164, 183, 274, 275, 298, 367, 382, 396, 399, 434, 435, 457, 458, 469, 470, 471, 477, 481, 486, 488, 491, 495, 496)
Martin Marietta Sodyeco, NC

2,4,6-Trinitrophenol, ammonium salt (Ammonium picrate; Picric acid, ammonium salt) (p. 89, 169, 396)

2,4,6-Trinitrophenol, sodium salt (Sodium picrate; Picric acid, sodium salt)
Hummel S. Plainfield, NJ
Northrop Asheville, NC

Trinitrophenolate (Lead picrate) (p. 94)

2,4,6-Trinitrophenyl ether (p. 30)

2,4,6-Trinitroresorcinol (Styphnic acid) (p. 15, 110, 157, 165, 167, 183, 495)
Northrop Asheville, NC
Olin East Alton, IL

Trinitroresorcinol, lead salt (Styphnic acid, lead salt) (p. 94, 97)
Remington Arms Bridgeport, CT

2,4,6-Trinitrotoluene (TNT; α-TNT) (p. 15, 16, 19, 28, 29, 33, 43, 45, 56, 58, 61, 70, 72, 73, 80, 81, 102, 103, 106, 108, 109, 110, 111, 113, 114, 115, 116, 117, 118, 126, 129, 130, 168, 169, 170, 171, 172, 173)

2,4,6-Trinitrotoluene (Cont'd) (p. 174, 175, 176, 177, 178, 183, 185, 186, 189, 235, 286, 287, 288, 289, 290, 291, 292, 314, 315, 316, 317, 340, 344, 345, 370, 383, 384, 393, 394, 396, 453, 454, 455, 456, 461, 462, 464, 465, 466, 484, 485, 486, 487, 495, 496, 497)

Trinitrotoluene (2,3,5-; 2,4,5-; 2,3,4-) (p. 19, 28, 70, 72, 495, 496)

2,4,6-Trinitro-1,3-xylene (p. 342)

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